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End Of Degree Thesis: A Study Protocol

Can a presurgical lymphogammagraphy aid in early detection of occult contralateral neck metastasis in contralateral cN0 T3-staged squamous cell tongue cancer non-midline invasive?

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List of Abbreviations

AJCC	American Joint Committee on Cancer
ALT	Anterior Lateral Thigh
cN0	Clinically negative neck
CEIC	Comité d'Ètica en Investigació Clínica
CT	Computerized tomography
CLNLD	Contralateral neck lymphatic drainage
CLNM	Contralateral neck metastasis
COX-2	Cyclooxygenase type 2
DOI	Depth of Invasion
EGFR	Epidermal Growth Factor Receptor
GP	General practitioner
H&N	Head and neck
H&E	Hematoxylin & Eosin
HSV	Herpes Simplex Virus
HDJT	Hospital Dr Josep Trueta
HVH	Hospital Vall d'Hebrón
HPV	Human Papilloma Virus
IHQ	Immunohistochemistry
LSG	Lymphoscintigraphy
MRI	Magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
NPV	Negative Predictive Value
OR	Operating Room
OCC	Oral Cavity Cancer
OCSCC	Oral cavity squamous cell carcinoma
PA	Pathological Anatomy
PPV	Positive Predictive Value
PET-CT	Positron Emission Tomography and CT
SE	Sensitivity
SLN	Sentinel Lymph Node
SLNB	Sentinel Lymph Node Biopsy
SPECT	Single Photon Emission Computed Tomography
SECOM	Spanish Society of Oral and Maxillofacial Surgery
ES	Specificity
SCTC	Squamous cell tongue cancer
Tc-99m	Technetium-99m-label
TC	Tongue cancer
VEGF	Vascular Epidermal Growth Factor

1 Abstract

BACKROUND. Squamous cell tongue cancer (SCTC) has a poor survival rate, especially when lymph nodes are involved. Detecting these nodes is challenging, and current imaging methods have limitations. Official guidelines lack clarity on treating advanced cases with clinically contralateral negative necks. We propose adding pre-surgical SPECT/CT Lymphogammagraphy and Sentinel Lymph Node Biopsy (SLNB) to the standard approach. These techniques have shown high accuracy, minimal risk, and precise anatomical imaging in other tumors and SCTC early stages, potentially improving the early detection and treatment of occult contralateral lymph node metastasis in clinically negative contralateral necks of T3 non-midline invasive SCTC.

OBJECTIVES. The primary aim of this study is to assess the sensitivity of pre-surgical SPECT/CT Lymphogammagraphy in identifying potential contralateral lymphatic drainage from the primary lingual tumor. In cases where contralateral lymph nodes are detected using this technique, participants will undergo a SLNB. Depending on the results, patients may become eligible for additional contralateral neck dissection. All participants will be monitored for a period of 2 years to track the progression of the cancer and to identify any instances of contralateral neck relapse, particularly in cases where the initial sentinel node study yielded negative results, affecting specificity and predictive values. Secondary objectives of the study involve evaluating the rate of contralateral neck metastasis within our sample and exploring the impact of initial N-stage and tumor size on the outcomes.

DESIGN AND METHODOLOGY. This study was designed as a longitudinal prospective 2-center study, oriented mainly for the principal objective. Secondary objectives will be assessed by a transversal design to confirm initial results (proxy data).

PARTICIPANTS. 118 patients diagnosed with T3-staged non-midline invasive squamous cell tongue cancer that show no contralateral neck metastasis in standard imaging techniques (CT/MRI). All patients must be eligible and in agreement for the corresponding surgical intervention.

KEYWORDS. Squamous cell tongue cancer; Oral cancer; Occult contralateral neck metastasis; Indication for contralateral lymphadenectomy; Sentinel Node Study; SPECT/CT Lymphogammagraphy; Sensibility; Specificity; false-positive rates.

2 Introduction

2.1 Epidemiology

According to *Global Cancer Observatory* (*Globocan*) 2020 statistics, oral cavity cancer (OCC) represents the 16th most incident type of cancer throughout all ages and sexes (377,713 new cases reported). Even though oral cavity carcinomatosis is most prevalent in Asia (65.8%), its' presence in Europe is not negligible. In our continent, oral cancer rises to 17.3%; being the second area with most prevalence worldwide, especially in men [Figure 1]. In 2020, there were 1.9 deceased individuals per 100.000 people (standardized by age) (1).

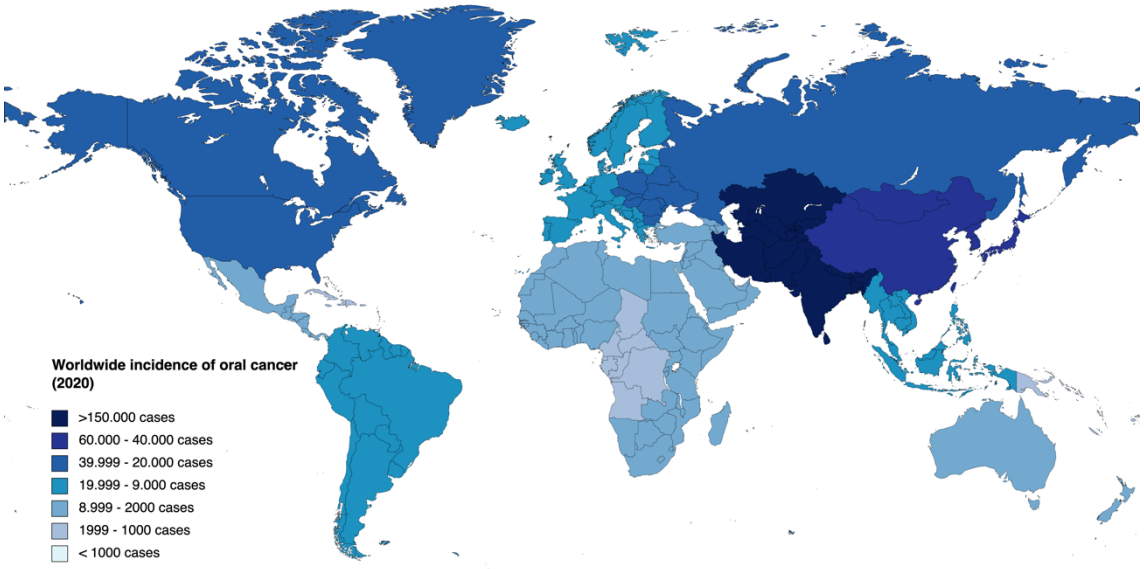


Figure 1. Map chart representing worldwide oral cavity cancer incidence in 2020. This chart shows weighted incidence distributed through the United Nations bioscheme. On a more global approach, Asia ranks first in incidence of OCC (65.8%) whereas Europe is found second (17.3%) out of the 377,713 cases reported in 2020. North America (7.3%), South and Central America (4.7%) and Africa (3.8%) have considerably lower incidences. *Based off Globocan data base for oral cancer (1).*

Excluding non-melanoma skin malignancies, OCC is the most common neoplasia of the head and neck (H&N). This area of neoplasms includes squamous cell carcinoma (90%),

minor salivary gland cancers and other exceptional tumors. The most frequent structures to be affected by this entity are the tongue (anterior 2/3[1]), lip and oral cavity floor (2).

Even though statistics for tongue cancer (TC) itself are limited, recent studies stated that there is a worldwide annual increase ranging from 0.4% to 3.3%, especially in our environment (3). This increased rate has been mainly observed in women, rather than men. In 2021, there were 8.188 new cases of oral cavity and pharyngeal cancer diagnosed in Spain as reported by the Spanish Society of Medical Oncology (4).

Despite the recent advances in therapeutics and early detection, TC still remains with a very poor 5 year survival rate at around 50% (2). The presence of cervical lymph node relapse greatly impacts in the survival rate; being associated to a prognosis decrease of 25% (5).

2.2 Etiology and major risk factors

There are multiple risk factors that have been thoroughly studied for oral cancer; toxic agents being the most classical and relevant (6).

This section reviews the most important predisposing factors will be exposed [Figure 2].

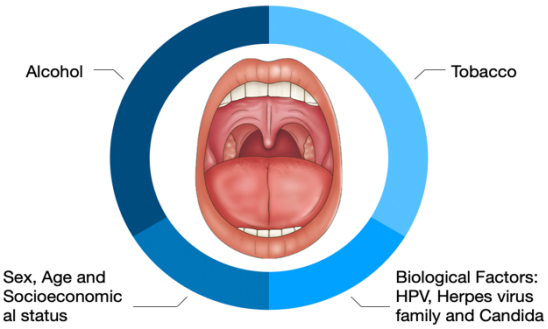


Figure 2. Predisposing factors for tongue cancer. Weighted diagram representing the main risk factors for TC.

Figure 3. Classic patient profile with tongue cancer. Illustrated representation of a smoker old man. *Extracted from iStockPhoto.*

[1] The anterior lingual two-thirds are considered part of the oral cavity. Meanwhile, the lingual base or posterior third is comprised as a part of the oropharynx. Therefore, it is generally not considered oral cavity carcinoma by itself, rather a larynx neoplasm.

Sex, age and socioeconomical status

Classically, lingual cancer has been a type of neoplasm most common in older smoker men [Figure 3]. Consequently, the male sex should have greater risk for TC. However, this tendency has had a sudden change during the last decades. Nowadays, studies have observed an increase in younger, white female patients. This epidemiological variation has been attributed to different factors, such as a female lifestyle change due to the progressively increased toxic substances usage (i.e., tobacco and alcohol) and unprotected sexual encounters[2] (7,8). Yet, this growth in female cases is mainly independent from toxic chemicals, which means that these individuals will not have a direct predisposing factor, like tobacco or alcohol. Unfortunately, little research has been conducted into this matter, leaving the question without response.

Nonetheless, studies have attested that OCC is six times higher in males than females (8).

The typical patient profile is, not only an older smoker (and usually alcoholic) man, but also someone from a low socioeconomical status. The reason why is because of the lack of access to correct oral hygiene, as well as late consultation (9).

Tobacco and alcohol

The main risk factors contributing to squamous cell tongue cancer (SCTC) appearance are prone to be tobacco, alcohol and other ambiance agents since there has been an increase in earlier life stages; which has also been observed in other western countries (10). Tobacco is harmful for the lingual and oral epithelium in all its states (cigarettes, cigars, pipe, hookah…) due to its high carcinogenic capacity in generating malignant-prone mutations.

[2] In oropharyngeal squamous cell cancer, there is a clear association with HPV infection. However, the oropharynx is not considered as part of the oral cavity.

Alcohol has been numerously suggested as major risk factor for OCC by facilitating cellular metabolism malfunction (6). Ethanol has shown a synergic effect combined with tobacco, not only affecting the ethylic daily dose but also to its carcinogenesis capacity. Therefore, OCC risk from alcoholic consumption is most pronounced among smokers; which will not only consume at a higher level but also have a higher chance of developing OCC (11).

Biological factors

Contrary to the classic oropharynx cancer, OCC has been weakly associated to Human Papilloma Virus (HPV), raising up to a 23.5% of cases (6). However, it has a strong connection to the classic factors [previously mentioned]. Some recent studies have shown presence of molecular evidence of HPV genome in OSCC tissues and its premalignant stages (i.e. oral leukoplakia), specially to HPV subtypes 16 and 18 (12). Despite the recent findings, HPV is more likely correlated to oropharynx originated tumors than OCC; the latest being less definite than the later (13).

Herpes simplex virus (HSV), in particular HSV-1, is commonly related to mouth and lip sores. As a result, it has been suggested as an ethiopatogenic factor for OCC. Other subtypes of HSV also present a potential carcinomatous relation to OCC; such as Epstein Barr Virus (EBV) and Cytomegalovirus (CMV) (14,15).

Candida *(C. albicans)* can generate squamous metaplasia with high proliferating activity, notably when in oral cavity fungal colonization. In spite of a need for extensive proof, clinical studies have observed infected *C. albicans* nodular leukoplakia having a tendency for dysplasia and malignant transformation, playing a enhancer paper in OCC origin (16).

2.3 Pathophysiology and histological examination of tongue cancer

Oral squamous cell malignancies origin from epithelial lining of the affected structure; in this case being the tongue. The tongue is a V-shaped muscular structure that plays an essential part in the oral cavity. The organ of speech and taste is divided into left/right

by a sagittal connective tissue septum (17). The middle line separating the halves is a vital component in the prognosis based therapeutic approach of its carcinomas.

Histological findings are crucial for diagnosing the common (90%) oral cavity squamous cell carcinoma (OCSCC) and other less common (10%) lingual neoplasia, such as basal cell carcinoma, adenocarcinoma, adenoid cystic carcinoma, and mucoepidermoid carcinoma (18).

The lingual epithelium undergoes molecular changes, progressing from normal to dysplasia and invasive forms. Tissue invasion may arise from isolated carcinomatous cells or epithelial mutated cell islands to a submucosal or bone malignant infiltration. Sometimes OCSCC might present itself in a exophytic variant in which the lesion margins appear erythematous in a "raised rolled" pattern (19). Specific changes in SCTC show basal membrane disruption and corium invasion with dyskeratosis, epithelial pearls and typical and atypical mitoses (20).

Studies suggest genetic and epigenetic factors contributing to the disease's appearance. The mutation of major tumor suppression proteins, p16 and p53, is no exception to H&N carcinomas. P16, encoded by CDKN2A, acts as an inhibitor of the Cyclin D1, a protein in the cell cycle that prevents phosphorylation and inactivation of pRb (another suppressor protein), specifically blocking G1 to S phase progression. Even though p16 is usually not mutated in OCSCC, its inactivation decreases the survival rate significantly (6,21).

Mutation of p53, encoded by TP53 gene, is commonly identified in 84% of H&N cancers, OCSCC being included. This protein plays an essential role in apoptosis or cell growth arrest. Despite being strongly linked to OCSCC tumor stage and grade, it has not been reported any connection to lymph node metastasis (21).

Other relevant genetic factors for OCSCC
○ Epidermal Growth Factor Receptor (EGFR): increased tumor aggressiveness and decreased survival rate in EGFR mutation. Present in 90% of H&N neoplasms.
○ PI3K/AKT/mTOR pathway: IHQ-tested pathway's implication in OCSCC.

○ NOTCH1: found in basal cells of oral squamous epithelium. Inactivated in OCC and oral epithelial dysplasia. Might partake as key in promoting OCSCC; being interconnected with clinical and T-stage. Yet, it is still being studied. ○ Wnt/β-catenin: this signaling pathway's upregulation leads to OCSCC's oncogenesis by a β-catenin aberrant activation. NOTCH1 inactivated tumors prevent inhibition of this pathway.

Table 1. Other relevant genetic factors for OCSCC. *Adapted from Husu PJ et al (21).*

Particularly regarding lymph node metastasis, cyclooxygenase type 2 (COX-2) and Vascular Epidermal Growth Factor (VEGF), subtypes C and D, expression has been found upregulated in most cases of OCSCC whereas high Ki67 index has been correlated to OCSCC tumors that developed distant metastasis (22). All the previous factors contribute to tumor lymphangiogenesis [Figure 4].

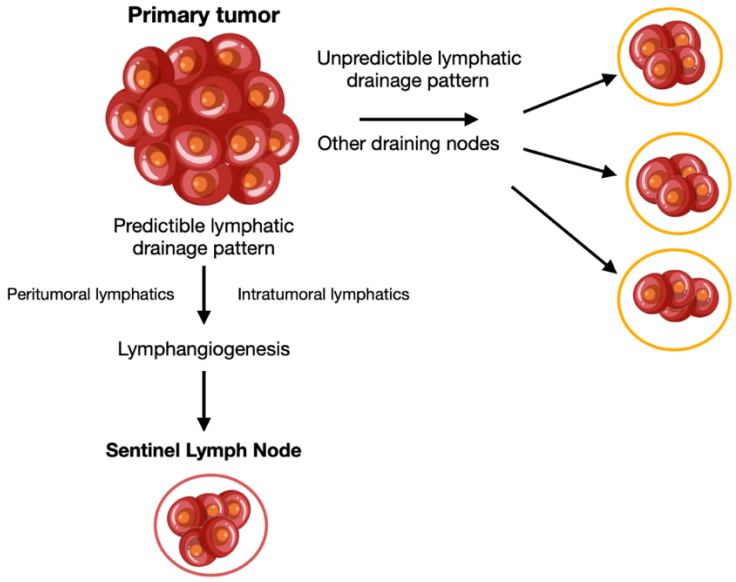


Figure 4. Pathophysiology of primary tumor's lymphatic spread. Lymph node metastasis arise from multiple molecular interactions regarding upregulated factors (i.e., VEGF) stimulating lymphangiogenesis. The first node that is affected by this extension of the primary tumor is the sentinel node. *Adapted from Sharma et al (23).*

One of the key components that ultimately leads to lymph node detection are cytokeratins. These structural cell support proteins are a part of the normal epithelium that get altered in squamous cells of OCC. Cytokeratins are targeted by immunohistochemistry (IHQ) monoclonal antibodies (AE1/3) for lymph node metastasis detection [see section 2.6.3 *"Imaging and histological results interpretation"]* (24).

Epigenetic genomic modifications are a major mechanisms underlying gene expression regulation mediated through its methylation and demethylation. The hypermethylated genes in OCSCC comprises cell cycle control genes, apoptosis, p73 and RAS, Wnt signaling pathway, cell adhesion and DNA repair genes (25).

2.4 Diagnosis

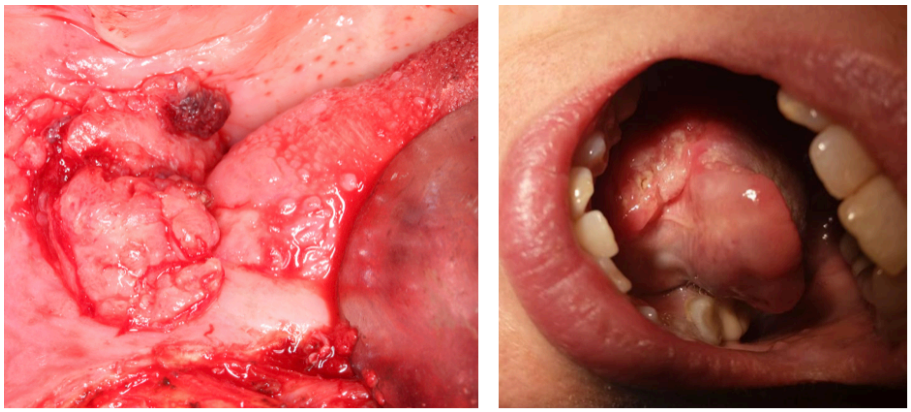
The diagnostic chain of TC usually commences as a patient consultation to their respective general practitioner (GP) or specialist due to an abnormality's appearance in the tongue.

During the medical visit, the specialist/GP starts by a thorough clinical interview about the patient's personal, family and pathological history, making emphasis in potential predisposing factors for TC (i.e., tobacco, alcohol…). Following the interview, the medical practitioner inspects and examines the tongue for lingual lining alterations with a light source; as well as cervical palpation for possible adenopathies. Characteristics stablished by the physical exam [Figure 5], correlated with the clinical portrait, will determine the need for further investigation.

Signs and symptoms that may indicate TC	
• Tongue and oral floor pain (most frequent) • Erythema • Leukoplakia	• Irregular-margined lesion • Rigidity to touch • Ulcers • Lumps

Table 2. Clinical manifestations of TC. *Based off Bagan J, Sarrion G et Jimenez Y (26).*

If there is a medium to high suspicion for a potential malignant process, lingual samples are taken to determine the nature of the lesion. That is performed by a small sized punch biopsy under local anesthesia. This sample collection usually consists of a single short cylindric sample extraction using a round-tipped tool. Afterwards, the doctor sends the sample to the Pathological Anatomy (PA) department [*see "Pathophysiology and histological examination of squamous cell tongue cancer"*].



A B

Figure 5. Lingual cancer presentation phenotypes. A. A 65 year old female presented an erythematous ulcerative irregular-margined tumor located in the tongue's insertion (close to the root); B. A 40 year old female presented an irregular-margined hypertrophic lesion in the right lateral margin of the tongue.

While the AP exam is processed, a widespread imaging study is requested which also includes H&N area and extension study (mainly of the thorax) to detect possible distant metastasis (M1). Magnetic resonance imaging (MRI) should be requested as the first imaging technique due to its high-performance in detecting soft tissue lesions. Computerized tomography (CT) and Positron Emission Tomography and CT (PET-CT) constitute tests that are usually left out for the extension study; even though CT might be indicated when in suspicion of cortical mandible invasion.

In presence of tongue cancer, the MRI study will show a T2 weighted fat-suppression hyperintense lingual area [Figure 6]. Tumor invasion visualization is usually preferred in coronal images whereas sagittal imagines are more useful for identifying neoplasms in the base of the tongue. TC frequently arises from the lateral and under surface and tend to locate near the midline or posteriorly. Dorsally located tumors are rare (27).

Even though CT has demonstrated higher performance in depicting cortical invasive lesions, MRI can indicate mandibular invasion as a low-signal intensity area with hyperintense medullary fat (28). Nonetheless, CT affiliates cortical invasion with high precision, which translates into an interruption or erosion of peripheral hyperattenuating of the mandibula rim (27) [Figure 6].

If there is presence of lymph nodes on presentation, the first areas that are usually colonized by malignant cells are the submandibular and jugulodigastric nodes, corresponding to level Ib and IIa respectively [Annex I]. Studies have thoroughly reported that oral cancer tends to disseminate through lymphatic networks most frequently nesting in levels I and II, even though other levels might be involved [Annex II]. Neck metastases would present as hyperintense nodular lesions throughout the cervical levels [Figure 6].

Since tongue cancer is a silent-progression cancer, it has been reported that 30% of patients might have occult neck metastasis that cannot be detected clinically or radiologically (27).

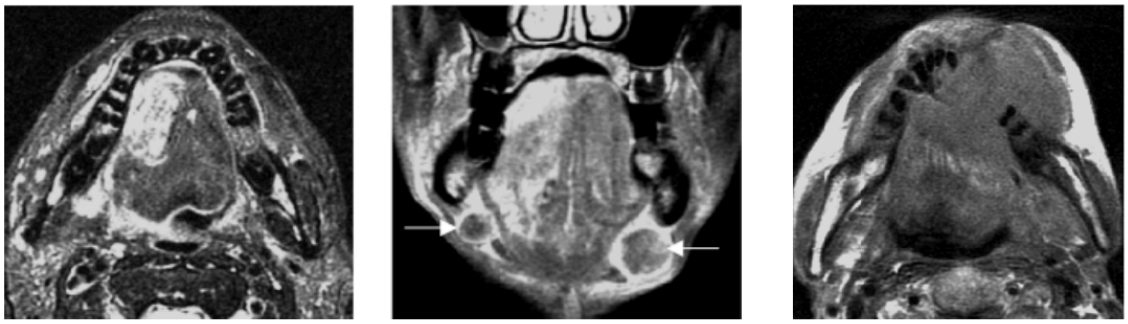


Figure 6. MRI studies of tongue cancer. A. Axial T2 weighted fat-suppression image shows a right-sided tongue cancer extending over 5 mm from the lateral margin of the tongue; B. Coronal T2 weighted fat-suppression image shows bilateral submandibular lymphadenopathy (arrows), a result of the lymphatic drainage pathways of the inner two-thirds of the oral tongue; C. Axial T1 weighted image shows a tongue cancer with mandible invasion. Extracted from (27,28).

This diagnostic protocol will finalize into the categorization of the case depending on the tumor size (T), lymph node presence and location (N) and distant metastasis (M) (TNM staging system). Patients will have different prognosis and therapeutic approach depending on the resulting TNM stage [Annex III].

Midline invasion and Depth of Invasion (DOI) play a key role in the cancer's prognosis. The higher the DOI and the closer to midline the higher the rate of cervical lymph node appearance [*see section 2.4.1 "Prognosis-related factors*"].

Detailing into CLNM, midline invasion has a greater weight of importance in its origin; therefore, CLNM are present when in lingual midline involvement. In these cases, patients undergo bilateral lymphadenectomy even though they may have clinically negative neck for CLNM (cN0).

As a TNM and related factors overview in this project, we focus on stage T3 which means that the primary lingual tumor is larger than 4 cm and has a depth of invasion of over 10mm but does not invade midline or deep structures (29).

The case is introduced in H&N Tumors Committee for a detailed personalized assessment regarding treatment by a multidisciplinary medical team. This kind of tumor committee is composed by radiologists, otorhinolaryngologists, maxillofacial surgeons, pathologists, oncologists and case management's nurses.

2.4.1 Prognosis-related factors

Midline invasion

In oral cancer, the most important prognosis factor is cervical lymph node involvement thought to be the key indicator for a poor outcome in SCTC.

The tongue's midline is a fibrous septum that is in close relation to a vast submucosal lymphatic plexus connected to cervical lymph node chains (30). This network communicates the tongue and the cervical area like a highway.
It has been demonstrated that midline involvement is a predictor factor for contralateral neck metastasis (CLNM). Consequently, if the primary tumor involves the lingual central septum, studies have reported a 65% of CLNM rates (30,31). This anatomical reference is objectified by MRI studies.

Depth of Invasion (DOI)

Depth of invasion is defined as the extent to which the cancerous cells have infiltrated the surrounding tissues from the primary tumor site. DOI is an important factor in the staging and prognosis of oral cancer.

A greater depth of invasion is often associated with a higher stage of cancer and a potentially worse prognosis. Accurate measurement of DOI is essential in planning the appropriate treatment for patients with oral cancer, reason why it has been included in the latest TNM edition [Annex III]. It is usually determined through clinical (i.e., MRI studies of the tongue) and pathological assessment and is a significant factor considered when assessing the risk and management of oral cancer.

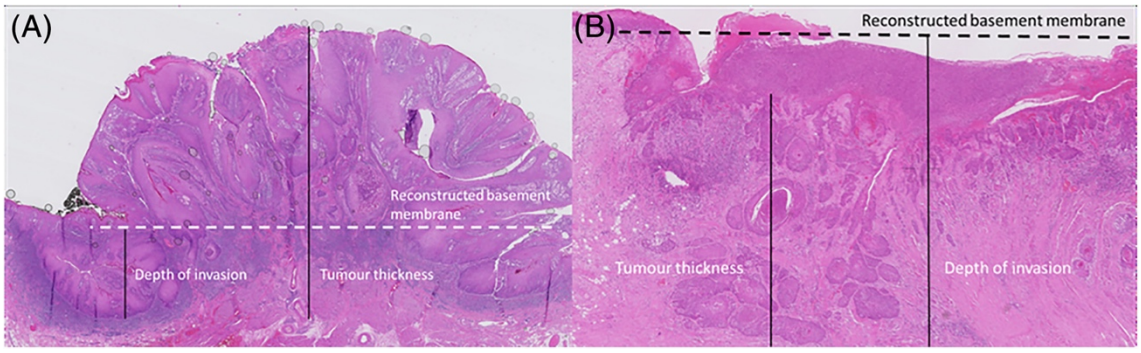


Figure 7. Measuring DOI from the deepest point of invasion. Reconstructed basement membrane line in exophytic tumor (A) and ulcerative tumor (B). *Source: den Toom, IJ et al* (32).

The definite measurement of DOI consists of histological examination that requires identifying the position of the basement membrane in the nearest normal mucosa and then extending a vertical line down to the deepest point of penetration (plumb line) (33), as shown in figure 7.

Histopathological grade

The histopathological grade is highly relevant for prognosis correlation. Essentially, the higher the grade, the worse prognosis for OCC. There are four types of degrees:

Grade 1 (G1)	Well differentiated. Cells seem normal and well differentiated.
Grade 2 (G2)	Moderately differentiated cells.
Grade 3 (G3)	Poorly differentiated.
Grade 4 (G4)	Undifferentiated.

Table 3. Histopathological degrees of cellular differentiation in OCC. *Based off Roi, A et al* (18).

2.5 Therapeutic outline

This section is a review of the current treatment outline based on the latest guidelines used in the clinical practice. The choice of treatment largely depends on the stage of the cancer, the patient's overall health, and individual preferences.

Adverse prognosis factors that greatly condition the adjuvant treatment decision are affected surgical margins, extracapsular infiltration, pT3-T4, N2-3, positive lymphatic nodes in levels IV-V and perineural, vascular or lymphatic invasion (34).

Surgery

- Primary Tumor Resection: Surgical removal of the primary tumor is often the initial step in treating SCTC. Depending on the size and location, a partial glossectomy (removal of a portion of the tongue), hemiglossectomy (removal of half of the tongue) or a total glossectomy (complete tongue removal) may be performed. Early staged tumors may beneficiate from surgery by itself, as long as if there is absence of lymphatic infiltration or adverse prognosis factors.

  Under general anesthesia, the resection is performed with MRI-projected images used in the operating room as a guide for removing the affected area. The resected lingual piece is then sent to the laboratory for its histological examination.

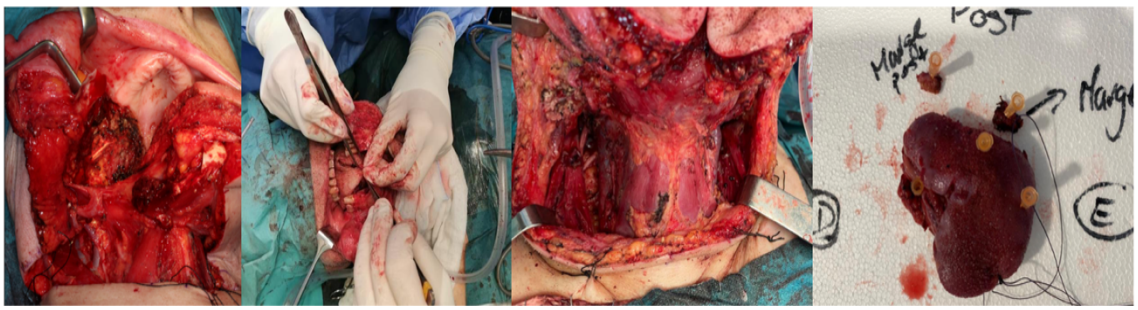


Figure 8. Lingual tumor and its resection. A. Partial glossectomy of lesion in image A, excision of part of the floor of the mouth and ipsilateral neck dissection; B. Hemiglossectomy initial incision; C. Bilateral lymphadenectomy of primary lingual tumor; D. Glossectomy's surgical piece that is sent to PA lab.

- Neck Dissection or Lymphadenectomy: In cases of clinically evidenced or high risk of lymph node involvement, a neck dissection may be performed to remove the affected nodes and assess the extent of metastasis.

  Neck dissections can be classified into radical and selective lymphadenectomies. A radical lymphadenectomy is an extensive neck dissection indicated when in presence of lymphatic metastasis (N+), implying the complete excision of all cervical levels (I-V) and adjacent structures (spinal nerve, sternocleidomastoid muscle, and internal jugular vein ligation); modified radical lymphadenectomy origins from the same concept but modifying the adjacent structures' dissection. Selective or elective lymphadenectomy, also labeled as functional, targets specific lymph node levels in clinically negative necks (cN0) depending on the risk of lymph node involvement, which ultimately relies on histopathological and anatomical factors [*see section 2.4.1. "Prognosis-related factors"*] (34).

  Cervical lymphadenectomies can also be classified depending on anatomical references according to the detected lymph nodes' location: Supraomohyoid (upper neck dissection), Anterior or Lateral, Posterior or Extensive neck dissection (34).

  The excised nodes and structures are submitted to the laboratory to be histologically examined with the lingual surgical piece [Figure 9][3].

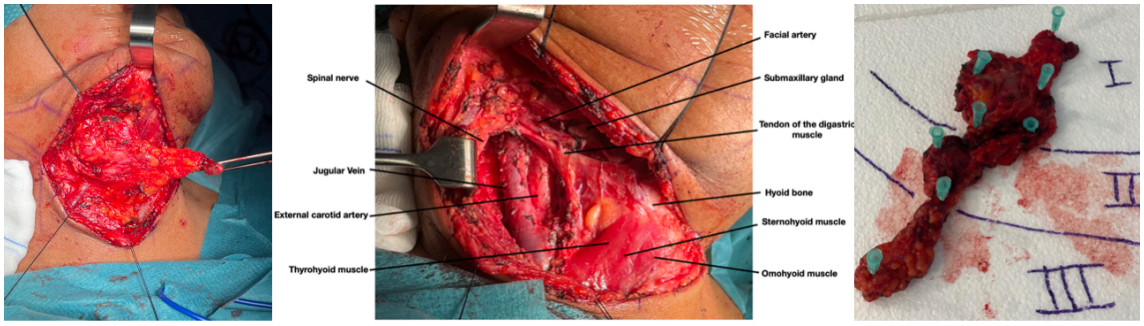


Figure 9. Unilateral lymphadenectomy. A. Elective unilateral neck dissection being performed showing great sized (very rare) adenopathies in already dissected levels IA and IB; B. Final image

[3] The PA examination consists of the surgical piece's fixation and embedding in formalin. Afterwards, it is section and stained with Hematoxylin and Eosin (H&E) for its microscopic observation.

after ipsilateral neck dissection of levels I, II and III with anatomical references; C. Presentation of the lymphadenectomy surgical piece.

- Reconstruction: Following surgical removal of considerable portion of the tongue, reconstruction techniques, including microvascular surgery, may be employed to restore oral function and aesthetics. These procedures aim to preserve speech, swallowing, and appearance.

  In our environment it is usually performed by using a radial forearm or anterolateral thigh free flap (ALT) that is fixed and re-vascularized through arteries dissected during the lymphadenectomy. A conduct between the floor of the mouth and the neck is left to connect the vascularization to the flap. Figure 10 shows a lingual reconstruction with an ALT flap, retrieved from the left thigh, that attaches to the right posterolateral lingual area (primary tumor location) and was anastomosed to the vascular dissected vessels during the right (ipsilateral) lymphadenectomy.

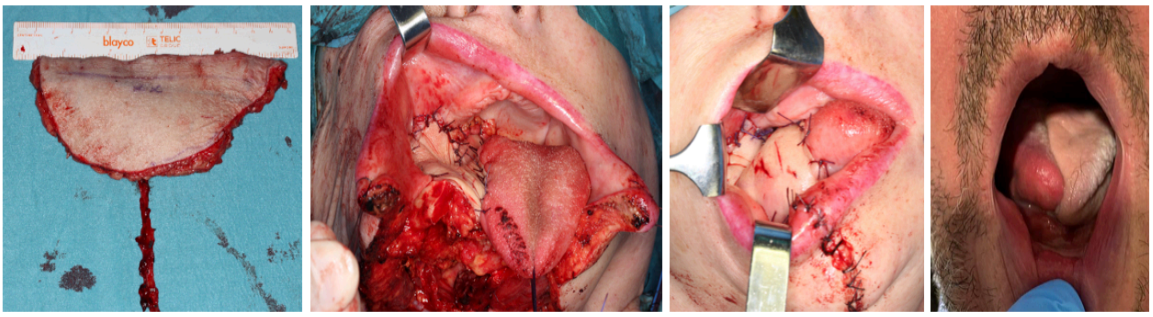


Figure 10. Reconstruction of tongue with ALT flap. A. ALT flap; B. Flap insertion to the recipient area and vascular anastomosis; C. Final result of ALT in place of where the tumor was located during surgery; D. Post-surgical visit that shows correct vascularized flap in a partially reconstructed tongue after an hemiglossectomy in a 58 year old male patient.

Radiotherapy

- External Beam Radiotherapy: A high-energy radiation source is used to target and eliminate cancer cells. Radiation therapy may be used as the primary treatment for small tumors (early stages – T1-2) or as an adjunct to surgery for larger or more advanced tumors.

- Brachytherapy: In some cases, internal localized radiation sources are implanted directly into the tumor or the surrounding tissues.

Chemotherapy

Adjuvant chemotherapy is indicated in regional or distant metastasis, which might be in combination with surgery or radiation. Neoadjuvant chemotherapy is administered before surgery or radiation to shrink tumors and make them more amenable to subsequent treatments.

Targeted Therapies and Immunotherapy

Emerging treatments such as targeted therapies and immunotherapies are being investigated for their efficacy in the treatment of SCTC. These therapies aim to target specific molecules involved in cancer growth and promote the body's immune system to fight cancer cells. Most are in research and not available in the normal clinical practice.

Supportive Care

Patients undergoing treatment for SCTC often require supportive care. This includes managing symptoms, such as pain, dysphagia, or speech problems. Speech therapy and dietary modifications are common components of supportive care.

Follow-Up Care

Regular follow-up appointments with healthcare providers are essential to monitor the patient's progress, address any side effects of treatment, and detect and manage any recurrence of the disease.

2.5.1 Treatment by stage overview

The following section exposes specifical treatment algorithms according to the tumor stage. All information has been extracted and adapted from the National Comprehensive Cancer Network (NCCN) Guidelines for H&N tumors, specifically for SCTC (34).

EARLY STAGED TUMORS

Early staged tumors (T1-2, N0) can be treated with surgery (glossectomy +/- neck dissection in positive sentinel lymph node biopsy) or radiotherapy (>70Gy to primary tumor +/- 50Gy to the neck) [Annex IV].

When surgery is elected as the first therapeutical option and when in absence of unfavorable prognosis factors, follow-up is indicated. Optional radiotherapy may be used if positive results of sentinel lymph node biopsy. Surgical resected tumors with unfavorable prognosis factors will require further treatment (re-excision, chemo-radiotherapy or, in selected cases radiotherapy). Surgery is favored over radiotherapy because it offers higher rates of cure.
Patients treated with radiotherapy as the first line approach that present complete response, follow up is indicated. In case of residual disease post-RT, surgical rescue is indicated.

LOCALLY ADVANCED TUMORS

Patients with larger sized tumors that do not invade deep structures (T3) and have a clinically negative neck (cN0) typically undergo surgery. The intervention bases on glossectomy with reconstruction and unilateral or bilateral selective neck dissection [Figure 11]. Patients without adverse factors might need radiotherapy.

Adverse features indicate further treatment (re-excision, chemo-radiotherapy or, in selected cases radiotherapy).

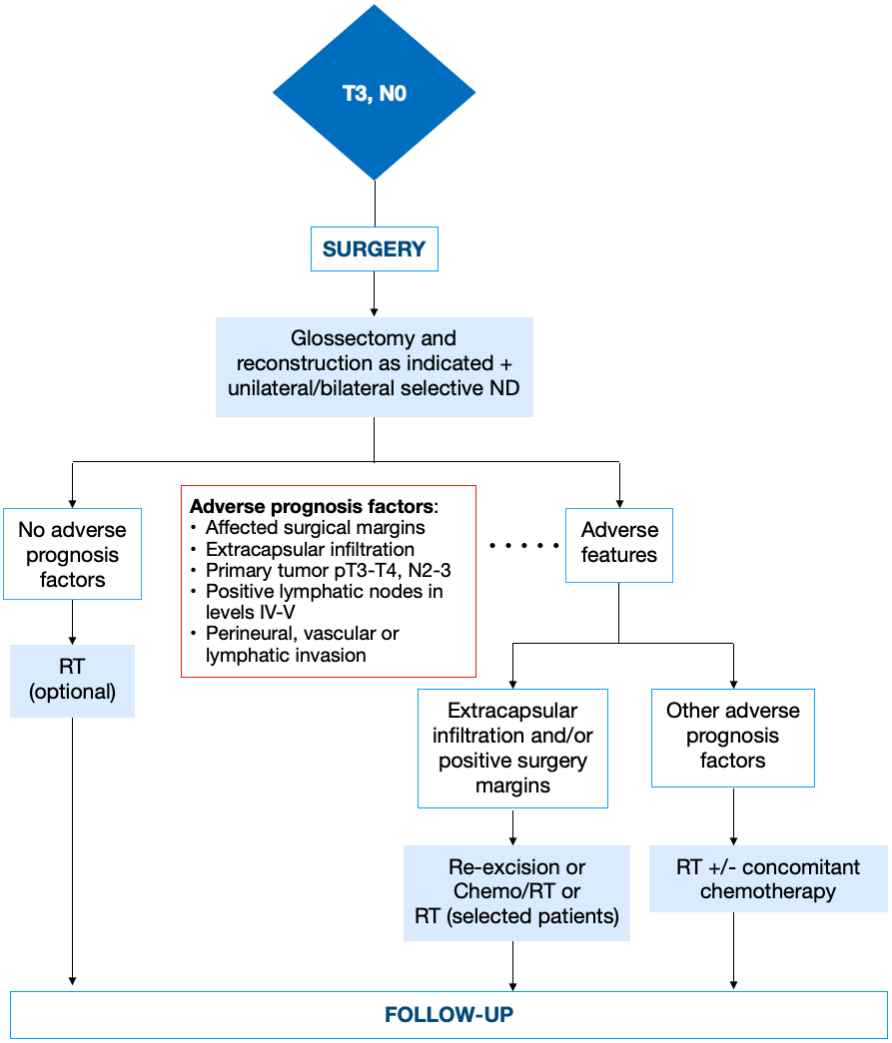


Figure 11. Locally advanced tumors treatment algorithm. – ND: neck dissection; RT: radiotherapy. *Adapted from NCCN.*

ADVANCED STAGED TUMORS

Surgically treated advanced cases (T1-3 N1-3 or T4a and any N) [Annex IV], undergo primary tumor's excision (glossectomy) and ipsilateral neck dissection +/- contralateral lymphadenectomy. Patients diagnosed with N2c will require glossectomy and bilateral lymphadenectomy.

Presence of adverse prognosis factors indicates for additional adjuvant treatment. When there is extracapsular infiltration or rupture and/or a positive surgical margin, combination of adjuvant chemo-radiotherapy is indicated. Other adverse factors might indicate radiotherapy and concomitant chemotherapy.

2.6 SPECT/CT Lymphogammagraphy and Sentinel Lymph Node Biopsy

2.6.1 Concept and foundations

In the developing fields of oncological care and diagnostic procedures, two remarkable techniques have arisen as pivotal tools in the precise assessment of cancer's extent and the facilitation of tailored treatment strategies.

SPECT/CT Lymphogammagraphy, often referred to as lymphoscintigraphy (LSG) or lymphatic mapping, and Sentinel Lymph Node Biopsy (SLNB) stand at the forefront of this advancement, offering profound insights into the management of cancer, particularly in cases where lymphatic spread is a concern. LSG is comprised as a Nuclear Medicine imaging technique whereas SLNB represents a surgical procedure rooted into the first technique's results.

In the current clinical practice, these techniques are indicated in early stages (T1-T2) of SCTC for early detection of ipsilateral cervical lymph nodes and its individualized treatment, as well as in breast cancer and melanoma.

2.6.2 Procedure

Lymphogammagraphy

SPECT/CT Lymphogammagraphy is a state-of-the-art imaging modality that seamlessly combines the functional accuracy of Single Photon Emission Computed Tomography (SPECT) with the anatomical precision of CT. This dynamic synergy enables medical professionals to visualize the lymphatic system with full clarity by administering a radiopharmaceutical tracer, specifically technetium-99m-labeled human serum albumin nanocolloid (Tc-99m), in four peripherally located points of the primary lingual tumor, each containing a 0,2mL volume (35–37).

The technique traces the drainage pathways using a two-headed gamma camera, eventually revealing the sentinel lymph nodes (SLN)[4] because of the

[4] Sentinel Lymph Node: initial lymph nodes to which carcinomatous cells are most likely to migrate.

radiopharmaceutical's accumulation [Figures 12 and 13]. The biological half-life of Tc-99m is approximately 24 hours, which allows for imaging procedures while keeping the total patient radiation exposure to a lower extent (36).

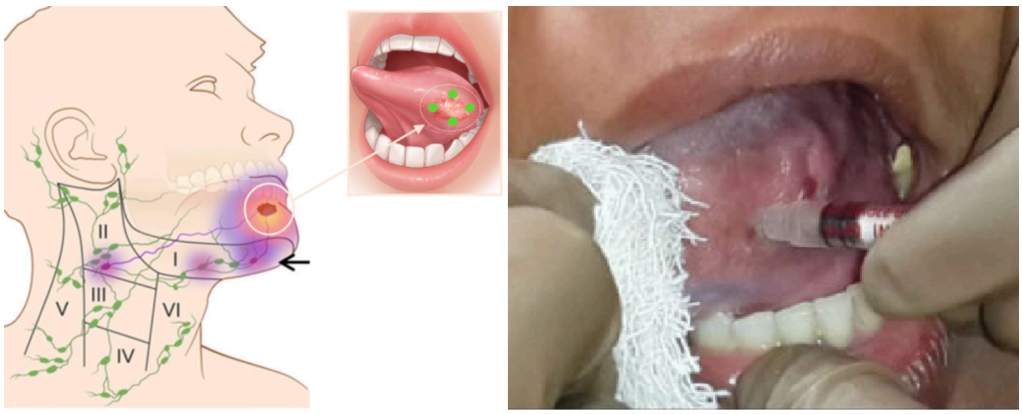


Figure 12. Lymphogammagraphy essentials. A. The process starts by administering the radiopharmaceutical in 4 peritumoral points (green circles). The primary tumor's radiation emission is more prominent and covers the hotspot of the sentinel lymph node located near the primary tumor (black arrow); B. Injection of radiotracer in lingual tumor under local anesthesia. *Adapted from University Medical Center Utrecht and Rathod R et al* (38).

The study of the lymphatic network should be performed pre-operatively, either the previous day to the surgery or the same day. In case of defectively depicted nodes, the imaging study is repeated the same day (after 2-4h) or on the same day of the surgery (39). This is done to ensure enough radioactivity during the making of the technique.

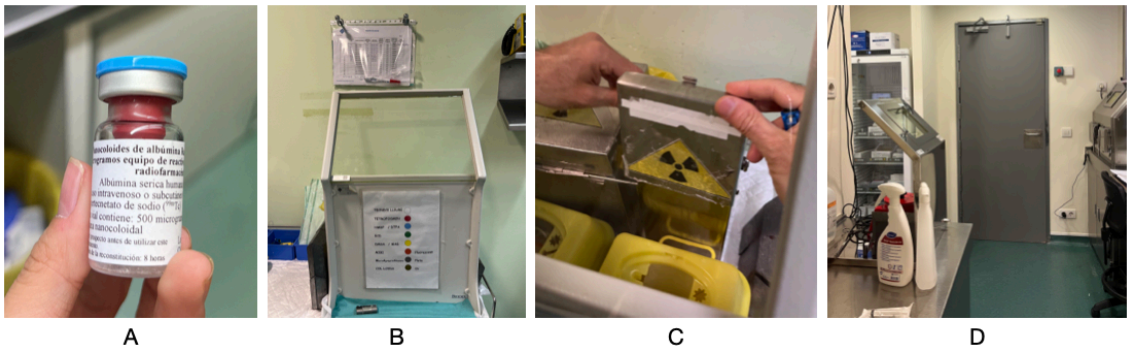


Figure 13. Radiotracer's characteristics and requirements. A. Albumin nanocolloid vial used in combination to the radiotracer; B. Field of work in which the nanocolloid particles are fused with

the technetium; C. Container in which technetium is preserved; D. Radio-pharmacy: place of storage of the radioactive pharmaceuticals.

According to the joint practice guidelines for radionuclide lymphoscintigraphy and the EANM guidelines for SLN localization in oral cancer, a large-field-of-view gamma camera provided with a high- or ultrahigh-resolution low-energy collimator should be used, with a 10%–20% window centered on the 140-keV energy peak of Tc-99m (35,39). The gamma camera is utilized on a supine-positioned patient to dynamically monitor the lymphatic drainage in the anteroposterior projection, capturing 1 image every 3 minutes (40).

When significant radiotracer accumulation is observed in the primary echelon node(s), dynamic imaging (duration: 5-10 minutes / 20-25s by frame / 1 image every 1-5 seconds) is halted. Static imaging is initiated obtaining images at 15, 30 and 60 minutes post-administration (300s) in different projections (anterior-posterior and lateral +/- anterior oblique when deemed necessary) which are crucial for attaining a comprehensive three-dimensional node localization (35–37,39) [Figure 14]. SPECT/CT provides high-anatomically detailed images which simplifies the detection of the SLN and the lymphatic capillaries filiation.

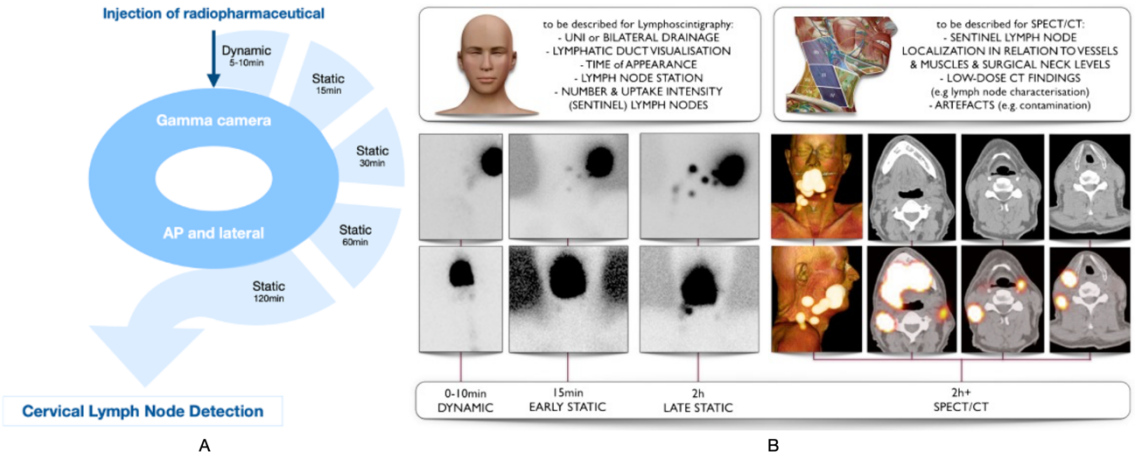


Figure 14. Lymphogammagraphic imaging studies. A. The study starts by a 5-10 minute dynamic phase continued by a static study that gathers images at 15, 30 and 60 minutes after the radiotracer's injection. *Adapted from SECOM (40);* B. Schematic imaging report generation with summary of interpretation criteria for LSG (right) and SPECT/CT fused images (left). *Extracted from EANM guidelines* (37).

Sentinel Lymph Node Biopsy

SLNB is an advanced surgical technique that draws upon the principles revealed by SPECT/CT lymphogammagraphy. It offers a minimally invasive yet highly accurate means of evaluating the SLN.

The SLNB bases from a 5 cm apron shaped surgical excision of the LSG-detected SLN and meticulously assessing these key nodes (41). The incision is particularly executed so that it allows for the neck dissection to be performed as a seamless extension of this incision, maintaining a natural flow. The SLN are identified by a hand-held gamma camera and marked on the skin with an indelible marker or blue dye. Once removed, SLNs are sent to the PA lab for its histological examination. They can also be double checked using the hand-held device after its excision.

The histological examination will determine the presence or absence of cancer spread in the lymphatic system or, in other words, lymphatic metastasis. The information gleaned from SLNB significantly influences treatment decisions, sparing patients from unnecessary extensive lymph node dissection while ensuring that appropriate therapeutic measures are pursued.

2.6.3 Imaging and histological results interpretation

Lymphogammagraphy

The dynamic study may reveal different foci of the radiotracer's accumulation. Determination of the SLN depends on the comparison of the dynamic images and the static study (35). The resulting Tc-99m nanocolloid accumulation in the lymph node is named as hot spot which objectifies the SLN. However, the filiation of the hot spot ultimately relies on the SLNB, which determines real metastatic involvement.

Subsequently, the nuclear medicine specialist and the maxillofacial or H&N surgeon observe the LSG joint images on a monitor for proper SLNB planification.

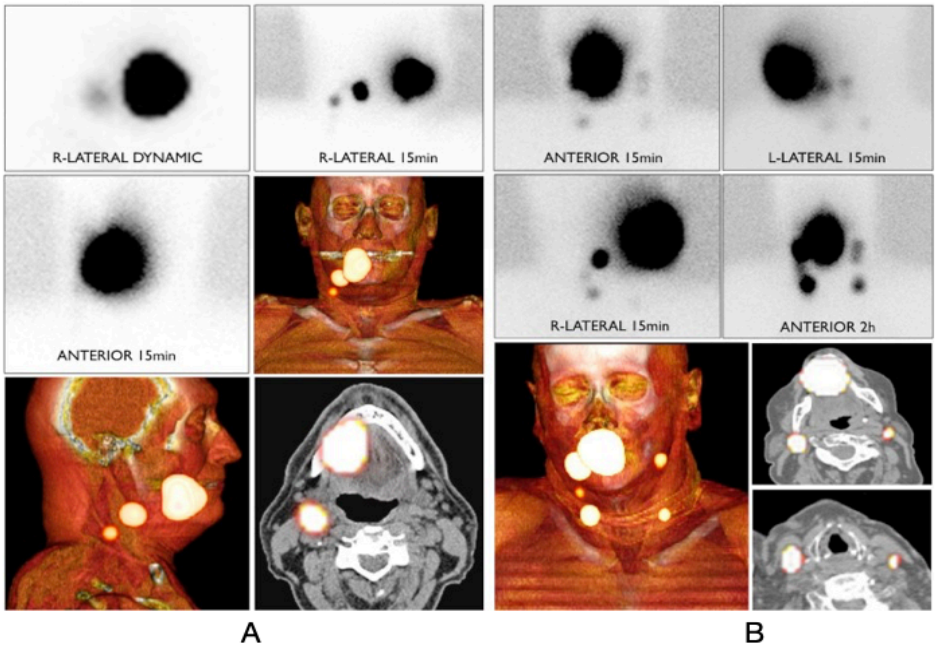


Figure 15. Example of SPECT/CT lymphogammagraphic study. A. Unilateral lymphatic drainage to the neck in a 55-year-old male patient with a T1 primary tumor localized on the right side of the tongue; B. Bilateral lymphatic drainage in a 72-year-old female patient with a T1 midline tongue carcinoma. *Extracted from the EANM guidelines* (37).

Sentinel Lymph Node Biopsy

SLN are submitted to PA laboratory as a fresh surgical piece while the patient remains in the OR. According to the *Spanish Society of Oral and Maxillofacial Surgery (SECOM)*, the histopathological exam consists in multiple sectioning and IHQ study that leads the phases exposed as follows [Figure 16] (40,41):

- Phase I: SLN are be fixed in a 10% formalin solution and divided into two parts either through the hilum if visible or along their midline. If the resulting halves exceed 2.5 mm in thickness, they will be further divided into additional 2.5 mm thick blocks. From each 2.5 mm slice, two histological sections will be prepared: one for hematoxylin and eosin (H&E) staining, and the other for staining with anti-pan cytokeratin antibodies AE1/AE3.

- Phase II: In cases of microscopical free-of-disease SLN, it will undergo mandatory serial multiple sectioning. The blocks will be cut at approximately 150 µm intervals and alternately stained with H&E and multi-cytokeratin antibodies (AE

1/3)[5]. Any positive IHQ findings must be confirmed by examining the H&E-stained sections.

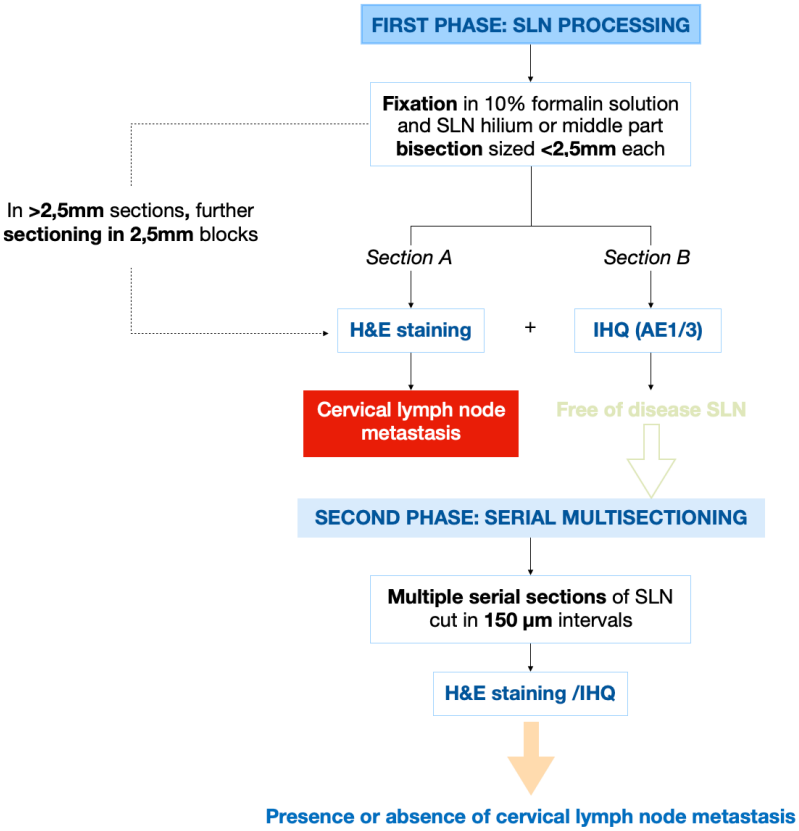


Figure 16. Histological examination of sentinel lymph node biopsy – SLN: sentinel lymph node; H&E: hematoxylin & eosin; IHQ: immunohistochemistry. *Adapted from SECOM.*

2.6.4 Contraindications

Lymphogammagraphy

SPECT imaging on its own generally does not have any absolute contraindications. However, patients may rarely experience allergic reactions to the tracer compound.

Healthcare providers should also exercise caution when considering SPECT for pregnant patients due to the potential risks of radiation exposure. Additionally, it's important to

[5] AE1 and AE3 are a pair of monoclonal antibodies used in IHQ to detect cytokeratin proteins. In OCC, AE1/3 can target multiple cytokeratins (1, 5, 10, and 14). These cytokeratins are commonly found in stratified squamous epithelial cells, which make up the lining of the oral cavity (24).

note that some obese patients may exceed the weight limit of the scanning equipment (42).

Sentinel Lymph Node Biopsy

SLNB in oral cancer comprises the following contraindications (23):

- Clinically positive neck nodes due to metastatic involvement, abnormal draining patterns may disrupt the typical lymph node architecture.
- History of previous surgeries or treatments that could have changed their natural lymphatic drainage patterns should be considered.
- Pregnant or lactating patients may require modifications to the extent of the SLN biopsy to minimize radiation exposure risk and the use of blue dye injections.
- Large primary tumors that can directly compress the draining lymphatics, special considerations may be necessary.

3 Justification

SCTC has a very poor 5-year survival rate (50%) that drastically descends when in presence of adenopathies (25%). Patients affected by this disease experience a worsening in their life quality because of aggressive surgical techniques; specially in advanced stage SCTC. Neck adenopathic presence is difficult to determine clinically, even with the recent imaging techniques. Despite diagnostic difficulties, hidden nodal metastasis rates of early-stage (T1-T2) SCTC have been reported up to 20% to 50% (43). It is only natural to assume that advanced stages (i.e., T3-T4) would have a greater chance of its development. In consequence, detecting them would impact in the therapeutic management in advanced cases of lingual cancer.

Neither the National Comprehensive Cancer Network (NCCN) or other official literature concerning oral cancer has a clear indication for the contralateral neck dissection in advanced cases with clinically negative necks. The NCCN, main reference for information about all cancers, does not provide a specific guideline for tongue cancer; but a compilation of all cancers related to the oral cavity. Nonetheless, it does state the bilaterality of its lymphatic drainage but its treatment left to preoperative clinical staging and the surgery's team judgement due to being a matter of great uncertainty (34).

Current standard imaging techniques (CT/MRI) for adenopathic detection cannot discern lymph nodes sized 10 millimeters or less and can leave over 30% of metastatic lymph nodes undetected (27,44). A 2019 meta-analysis determined low sensitivity and moderate specificity of F18-FDG PET/TC for cervical lymph node detection of H&N SCC, even considering its substantially higher cost in comparison to other imaging tests (45).

Regarding the therapeutic approach, even though we might encounter advanced stage tumor (T3), when midline invasion is absent, there is no indication for complementary treatment for the contralateral neck; meaning that the patient will only receive ipsilateral surgery and radiotherapy when their contralateral cervical area might have HCNM.

SPECT/CT LSG complimented with SLNB to detect potential sentinel nodes in the contralateral neck area would be a key additional diagnostic chain to compliment the actual standardized protocol. This Nuclear Medicine and surgical combination of techniques are very well studied and used in multiple cancers, such as breast cancer, melanoma and early staged oral cancer. This techniques have reported a wide range of sensitivity (70-100%) and false negative results (2.56-36%) (46–49) in other type of tumors, partially meliorated by high definition images from SPECT/CT LSG (50). Yet, most of this broad range in diagnostic validity values can be attributed to an underexperienced operator, which in our environment is rather unusual.

The Sentinel European Node Trial (SENT) are a consensus guideline recommendation for early staged OCSCC that have not shown cervical lymph nodes in the physical evaluation or in imaging techniques; or in other words, clinically and radiologically negative (cN0) cervical lymphatic chains. Even though these guidelines have defined SLNB as a standard of care, its implication has been just proven in earlier OCSCC stages (I-II) whilst leaving advanced stages into controversy (III-IV) (51,52), especially for advanced stage SCTC that does not compromise the lingual midline. Especially in great sized invasive tumors, it has been observed in some cases that lymphatic capillaries are obstructed preventing the radiotracer's trajectory through them. That is the main reason to indicate this technique in staged T3 non-midline invasive tumors, since the size is limited to small-medium tumors without deep invasion.

Moreover, it should be noted that the SENT findings demonstrated that T1-T2 SLNB-staged OCSCC patients could potentially avoid undergoing a neck dissection in 70% of cases. Additionally, the trial reported an impressive 3-year disease-free survival rate of 92%, a statistic that escalates to 95% among patients who tested negative for SLN (53).

Considering all the previously mentioned factors, we suggest adding a pre-surgical SPECT/CT LSG and SLNB to the current standard of practice to have an early approach into occult lymph node metastasis's treatment with high performance values, low risk-associated complications, direct pre/intra-surgical study, and anatomical imaging precision.

4 Hypotheses

4.1 Main hypothesis

For individuals with T3 non-midline invasive squamous cell tongue cancer with clinically negative contralateral neck, the detection of a pre-surgical contralateral neck SPECT/CT lymphatic mapping drainage pattern suggests a greater likelihood of confirmed-SLNB occult contralateral neck metastasis, exhibiting superior diagnostic performance (sensibility).

4.2 Secondary hypotheses

- The pre-surgical lymphogammagraphic absence of contralateral neck drainage demonstrates high specificity, positive and predictive values indicating the absence of contralateral cervical adenopathies. We aim to validate this hypothesis by using follow-up data as a proxy for confirmation.
- A significant proportion of cases involving T3 non-midline invasive squamous cell tongue cancer exhibit contralateral neck gammagraphic drainage patterns.
- In cases with higher-grade lymph node stages (N+), there is a higher incidence of drainage patterns compared to cases with lower-grade staging.
- Larger tumor sizes, there is a higher rate of contralateral cervical lymphatic drainage compared to smaller tumor sizer.

5 Objectives

5.1 Main objective

The principal aim of this study is to evaluate diagnostic validity (sensibility) of pre-surgical SPECT/CT lymphogammagraphy in patients with contralateral cervical lymphatic drainage (CLNLD) of clinically negative contralateral necks of T3 non-invasive midline SCTC as to determine presence of hidden contralateral neck metastasis, confirmed by histological examination.

5.2 Secondary objectives

- Calculate specificity, positive and negative prediction values under the assumption that the absence of contralateral neck gammagraphic drainage results indicate the absence of contralateral cervical adenopathies, and validate this assumption using follow-up data as a proxy.
- Determine the percentage of our population that show SPECT/CT LSG lymphatic drainage in the contralateral neck in comparison to the appearance of CLNM during follow up.
- Observe if there are differences of lymphatic drainage patterns between different N-staged SCTC.
- Examine if there are differences of lymphatic drainage patterns between different T-staged SCTC.

6 Methods

6.1 Study design

This project is meant to be designed as a **longitudinal study** that **transversally evaluates secondary objectives**, particularly focusing on specificity and prediction values across different time points during follow up.

This protocol is designed for use exclusively at *Hospital Doctor Josep Trueta* (HDJT) in the province of Girona (Spain), and *Hospital Vall d'Hebrón* (HVH) in the province of Barcelona (Spain), making it a 2-hospital multicenter study with a specific focus on their Maxillofacial Department.
These third-level medical assistance hospitals will provide the sample as well as follow the Guidelines for Squamous Cell Tongue Cancer that are defined in this project. The surgical team is thoroughly experienced in the target surgical technique (ipsilateral or bilateral neck dissection) and the primary tumor's surgical intervention (hemiglossectomy).

Our study will involve assessing the presence or absence of contralateral neck lymphatic drainage in patients affected by SCTC staged T3 that does not cross the tongue's midline. This will be examined through SPECT/CT lymphogammagraphy performed prior to the standardized surgical intervention for contralateral N0-staged neck (hemiglossectomy +/- neck dissection).
Individuals that show presence of contralateral neck lymphatic drainage will undergo intraoperative SLNB, whereas in absence they would be omitted for additional tests or therapeutic options for the contralateral neck. Therefore, this study does not include randomization in its design.

Intraoperative SLNB samples will be sent for a histological examination to assay the presence or absence of tumoral cells. If the SLNB is negative for malignant cells, patients will be omitted for contralateral neck dissection and will follow through standardized

surgical intervention. Additional surgical treatment (contralateral lymphadenectomy) will be performed in individuals who show positive SLNB.

Subsequently, the lymphadenectomy will also be submitted to AP for a further histopathological examination.

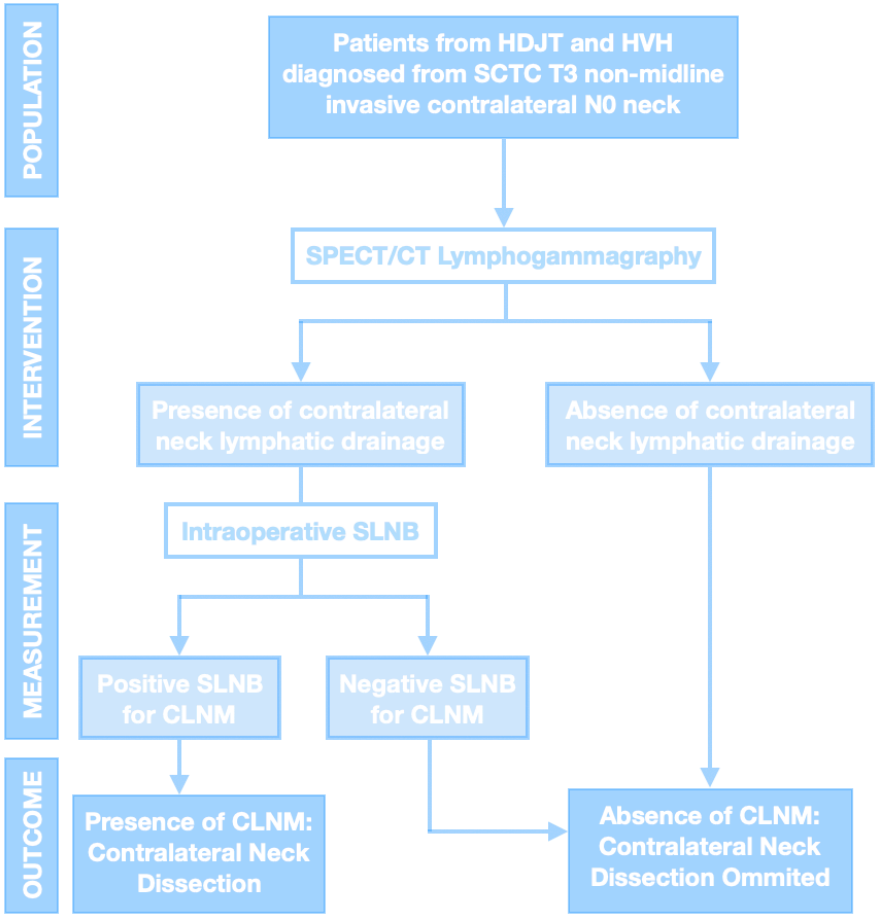


Figure 17. Outline of study design. *HDJT: Hospital Dr Josep Trueta; HVH: Hospital Vall d'Hebrón; SCTC: squamous cell tongue cancer; SLNB: sentinel lymph node biopsy; CLNM: contralateral neck metastasis.*

6.2 Study population

The target population of this study will be men and women included in HDJT and HVH census (Catalonia) diagnosed with SCTC staged T3 that does not involve the tongue's midline, eligible for surgery, that do not present contralateral neck metastasis at the time of the study (contralateral cN0).

All patients must fulfill the following inclusion and exclusion criteria:

Inclusion criteria	Exclusion criteria
• Age >18yo • No other previous cancer history • SCTC T3 non-midline invasive with/without ipsilateral adenopathies • Absence of contralateral cervical adenopathies in previous imaging studies (TC/MRI or PET-TC) • Absence of distant metastasis (M1).	• Not eligible for surgery • Previous history of tongue cancer • Contraindications for gammagraphic studies • Contraindications for sentinel lymph node biopsy • Known neck related pathology that can test as falsely positive • Rejection of treatment • HPV positive

Table 4. Inclusion and Exclusion criteria for population sampling.

6.3 Sampling

6.3.1 Sample selection

The sample will be obtained through a consecutive non-probabilistic sampling. The possibility of entering the study will be offered to patients who have been diagnosed with T3-staged SCTC without lingual midline involvement and contralateral cN0 willing to undergo its surgical approach.

All patients included in the study at HDJT and HVH that fulfill the inclusion criteria will be asked to participate and will be yielded the information document and the informed consent. Healthcare professionals will underscore the voluntary and confidentiality matters of patients' participation.

6.3.2 Sample size

In pursuit of attaining a statistical significance level with an alpha risk of 0.05 and a beta risk below 0.2 in a two-sided comparison, it is necessary to have a total of 118 subjects. This sample size is required to detect a statistically significant difference between two

proportions. Traditionally undiagnosed contralateral neck metastasis by standard imaging techniques in our environment is 30% (27). Early staged oral tumors that undergo this diagnostic procedure have reported high rates of accuracy ranging from 70-90%. Therefore, we estimated that in order to consider the clinical correlation statistically significant, the technique would have to detect at least 50% of the 30% undetected neck contralateral metastasis (15% of 30%) (51,54).

The estimation assumes a 10% loss to follow-up, mainly attributed to death, even though the main outcome of study would be measured during the patient's treatment process. The ARCSINUS approximation method has been applied for the sampling approach through paired measurements with **GRANMO software**.

6.3.3 Estimated time of recruitment

The selection of the target population will be retrieved in **2 years**. Currently, HDJT diagnoses around 24 cases of T3 staged SCTC every year whereas HVH diagnoses approximately 45 cases. Therefore, there is a 2-year estimated time for recruiting patients with SCTC that does not surpass the lingual midline.

6.4 Variables and measurement

6.4.1 Study variable

Contralateral cervical lymphatic drainage

The presence of potential cancerous lymphatic drainage of contralateral neck of primary SCTC staged T3 that does not invade the lingual midline is our study's variable. Our assumption would be to relate this abnormal drainage to induced contralateral malignant lymph nodes, secondary to the tumor.

Consequentially, this study's variable measurement will be conducting a SPECT/CT lymphogammagraphic study prior to the standardized surgery in patients diagnosed from T3 non-invasive midline SCTC that, therefore do not present contralateral neck

metastasis prior to the imaging study. The LSG is based on a Tc-99 sulfur nanocolloid radiotracer processed by a SPECT/CT scan [*see section 6.5 "Measuring instrument of main variables"*].

It would be defined as a **binary qualitative variable**:

- Presence of lymphatic drainage in contralateral cervical area.
- Absence of lymphatic drainage in contralateral cervical area.

6.4.2 Outcome variable

Contralateral neck lymph nodes

The contralateral cervical relapse is the main variable of this study. This variable would be measured by histological examination of the intraoperative SLNB to determine the cellular nature of the contralateral lymph nodes found in SPECT/CT LSG. See section 6.5 for further information about the SLNB as a measuring instrument *["Measuring instruments for the main variables*"].

Additionally, individuals that show histological infiltration of the intraoperative SLNB will undergo bilateral lymphadenectomy, which would be an additional measurement of performance.

It would be defined as a **binary qualitative variable**:

- Histologically confirmed contralateral neck adenopathies.
- Histologically confirmed contralateral neck free of adenopathic disease.

	Variables	Description	Measurement	Categories
	Contralateral neck		SPECT/CT Lymphogammagraphy	- Presence of contralateral cervical lymphatic drainage.

Study variable	**lymphatic drainage**	Qualitative nominal dichotomous		- Absence of contralateral cervical lymphatic drainage.
Outcome variable	**Contralateral neck lymphatic metastasis**	Qualitative nominal dichotomous	Histological confirmation by surgical specimen	- Presence of CLNM - Absence of CLNM

Table 5. Description of the main variables included in the study. – CLNM: contralateral neck metastasis.

6.4.3 Secondary outcomes

- **Tumor size in stage T3**: continuous quantitative variable starting at 4 cm. It is measured by histologic diagnostic criteria.

- **Ipsilateral lymph nodes staging at diagnosis**: ordinal qualitative variable measured by TNM staging of lymph nodes (diagnostic criteria) that is categorized by N0/N1/N2a/N2b.

	Variables	**Description**	**Measurement**	**Categories**
Secondary variables	**Tumor size**	Quantitative continuous	Histologic diagnostic criteria	Starts at 4cm
	Ipsilateral lymph nodes staging	Qualitative ordinal	TNM staged diagnostic criteria	N0 / N1 / N2a / N2b

Table 6. Description of secondary variables.

6.4.4 Other variables

There are other variables that can vary the diagnostic validity parameters due to its influence on the main variables of the study. Therefore, they should be partaken to evaluate their relation.

- **Age**: quantitative continuous variable, expressed in years.
- **Sex**: nominal qualitative variable expressed by male/female.

- **Education level**: qualitative ordinal variable expressed by high/medium/low and measured by degree of studies (ISCED scale) [Annex III].
- **Tobacco**: polytomous nominal qualitative variable expressed by smoker/non-smoker/former smoker.
- **Alcohol**: dichotomous qualitative variable expressed by yes/no.

Variables	Description	Measurement	Categories
Age	Quantitative continuous	Self-referred	
Sex	Qualitative nominal dichotomous	Self-referred	Male / Female
Education level	Qualitative ordinal	Self-referred according to ISCED scale	High/ Medium / Low
Tobacco	Qualitative nominal polytomous	Self-referred	Smoker / Non-smoker / Former smoker
Alcohol	Qualitative ordinal	Self-referred and categorized by SBU	No risk (0SBU) / Low risk (♂17SBU; ♀11SBU) / High risk (♂ 17-28 SBU; ♀ 11-17 SBU) / Dangerous consumption (♂>28 SBU ; ♀>17 SBU)

Table 7. Description of the other variables included in the study. – SBU: standard beverage unit.

6.5 Measuring instruments of the main variables

6.5.1 SPECT/CT Lymphogammagraphy

The LSG machinery consists of a double-composed imaging machines that combine the SPECT (nuclear medicine image) and the CT (regular imaging technique). The CT scan is coupled behind the SPECT, so the patient first undergoes the SPECT and, is afterwards displaced through the CT scan using the railed gurney.

Even though the SPECT consists of a two-phase study (dynamic and static), the main phase of interest for us is the static phase in which the imaging images will be fused with the CT images to dispose of a high anatomical resolution. The SPECT cameras rotate around the patient to get the different projections. The dynamic phase is the shortest (5-10 minutes after Tc-99m injection) and, in our case, it is the first view of the lymphatic drainage but does not provide key information about the potential sentinel lymph nodes. Yet, in our study, the main phase is the static phase which will be based on capturing frames at 60 and 90 minutes after Tc-99m injection and, if Tc-99m migration is detected, it proceeds to the fusion of SPECT/CT images. The final images will reveal the SLN-suggestive hot spots.

HDJT possesses two SPECT/CTs, a *Siemens Symbia Pro* and a *Discovery NM/CT 670* SPECT/CT [Figure 18]. Both machines have the same characteristics, so equivalent performance is to be expected. HVH also counts with similar machinery that will be programmed with the standardized specifications to ensure consistent performance between the two participating centers.

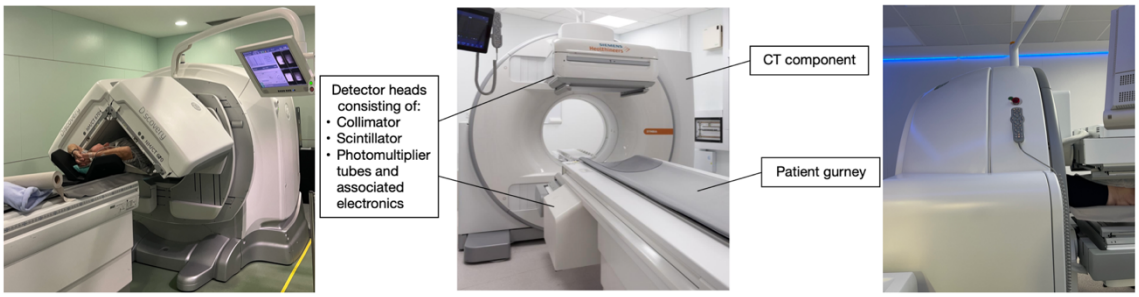


Figure 18. SPECT/CT machinery in our study. A. *Discovery NM/CT 670* SPECT/CT being used: the machinery surrounding the top of the patient (gamma camera) rotates to capture images in the diverse planes; B. front view of the *Siemens Symbia Pro* SPECT/CT with its components; C. *Siemens Symbia Pro* SPECT/CT (profile) to observe closeness of the detector heads to the patient (studied for breast cancer).

Our study's radiotracer (Tc99m nanocolloid) will be infused into four different peritumoral points of the lingual lesion. Each peritumoral point receives a 0.2 mL

injection of Tc99m, administered with local anesthesia. Patients will wait for 90 minutes to ensure the tracer's drainage through the lymphatic capillaries.

The imaging parameters for an adequate SPECT image acquisition are set at 140keV at a preset time of 120 to 300 seconds. The SPECT/CT image acquisition is made at a circular orbit with a two-headed camera to retrieve a 20-30 seconds per view image.

6.5.2 Sentinel Lymph Node Biopsy

The sentinel node is surgically traced with *Dilon technologies*' handheld gamma camera device model Navigator™ 2.0 after the radiotracer's administration. This Gamma Probe System comes with different types of compatible gamma probes that can be sterilized [Figure 19]. Its energy detection range can be set from 0 to 650 keV and must be set in "Scan" to start the mapping.

In our study, the device will be programmed with the Tc99 setting which detects its activity to 140keV. The detection of the potential SLN requires a 14mm or 11mm standard sentinel node mapping probe since it will be done on the patient's tissues during surgery.

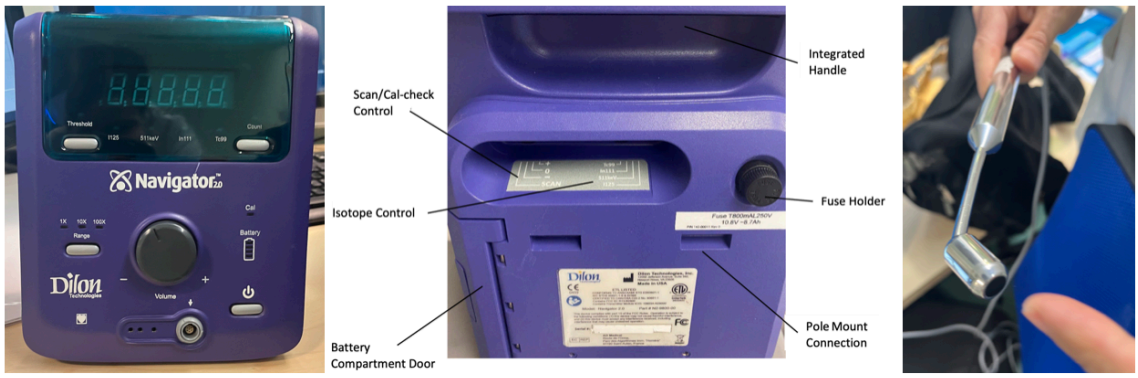


Figure 19. Navigator™ 2.0. A. Navigator control unit that translates the tracer's signal captured by the pilot probe to a sound notice that translated as the observed hot spot in the LSG; B. control unit features (rear): "scan" must be on to start with the mapping; C. 14mm standard handheld pilot probe: The finger indicates the gamma probe's tip through which the radioactivity is registered.

The SLN excision will consist of the excision of the signaled lymph nodes by the gamma probe. The patient will be fully anesthetized during the whole intervention. An apron-shaped incision will be performed on the patient's neck which might be taken advantage of in case of needing the lymphadenectomy. The nodes will be selectively excised and sent to the PA laboratory.

Foremost, the histological examination of the biopsied hot spots will be carried out as stated by protocol [*see section 2.6.3 "Imaging and histological results interpretation"].*

6.6 Safety

SPECT/CT lymphogammagraphy is considered safe, as well as SLNB, and has been used in multiple studies and in the clinical practice. Hence, it has proven acceptable safety profile. Nevertheless, mild adverse events might occur. The principal risks of this technique include:

- Mild hemorrhage, discomfort, or localized edema at the site of needle insertion.
- Allergic reaction to the radiotracer (extremely rare).

SLNB is a technique substantially less invasive than surgical lymphadenectomy. As a result, SNLB is generally used to avoid unnecessary lymph node dissection. Yet, it is not without its share of adverse events, the most common of which include the following (55):

- Lymphedema
- Wound infection
- Seroma development
- Discomfort

Rarely (<1%), injuries to the facial and spinal accessory nerves are possible through minimal exposure in SLNB (39).

Note: Nor SPECT/CT LSG or SLNB techniques have not been associated with any long-term negative health effect regarding radiation risks due to the low dose used in these studies.

6.7 Data collection

The initial stage of this study involves establishing a computerized database using SPSS software to facilitate effective information gathering. The information will be retrieved from target profile-compatible patients diagnosed in HDJT and HVH. Data collection will

strictly adhere to protocols ensuring confidentiality and anonymity. For this purpose, the identification number will be used to code the participants' names and personal information.

Figure 20 collects an overview of the patient journey whereas Table 8 resumes the data collection process.

First visit

Our study initiates with patients diagnosed of squamous cell tongue cancer staged T3 that have not shown midline involvement and lack of clinical contralateral neck lymph nodes (cN0) assessed by imaging studies (CT and MRI). These individuals will be included in the H&N Tumors Committee with the diagnosis' confirmation. Patients and their families will be informed of these findings and presented with their therapeutic options. Patients who satisfy all the inclusion criteria and do not meet any of the exclusion criteria will receive an invitation to participate if they are willing to undergo surgical treatment.

During this invitation, the study's characteristics and objectives will be communicated to the patient. To wrap up the appointment, a second visit will be arranged for the following week to allow the patient ample time to make their decision.

Second visit

If the patient agrees to participate, they will be provided with the Informed Consent Document and the Participant Information Sheet [Annexes VI and VII]. This medical appointment will consist of various points:

a. *Review and contrast the clinical history registered in the hospital clinical data base (SAP).* This will facilitate the retrieval of the rest of the variables, as well as complementing the previous known data from the patient.
b. *Extensive general and oral physical exam*. Oral mucosa needs to be examined, as well as the cervical area, for thorough confirmation of the primary tumor's state and its' adenopathic dissemination.

c. *Preoperative imaging study (complementing the imaging study if necessary)*: Further imaging studies might be required in some cases that were not performed in the first visit (i.e., extension study), explicitly as the preop study.
d. *Patient's and family doubts and worries*.

Third visit: Lymphogammagraphic study and preoperative examination

This visit will consist of routine preoperative examination, directed from the Anesthesia Department, to determine the patient's eligibility to be intervened. In absence of contraindications, the SPECT/CT lymphatic mapping will be executed.

SPECT/CT LSG will be performed as a preoperative test a day before or the same day of the surgery in accordance with the technique [*see section 2.6.2 "Procedure"].* If performed the previous day, the radiotracer's radioactivity must be considered to assess re-administration of Tc99m. The Nuclear Medicine department at HDJT will serve as the reference for conducting the lymphogammagraphy. The results will be coded with the patient's encrypted ID that was initially created.

Sample collection: Intraoperative SLNB and surgical intervention

Patients that showed lymphatic drainage in the contralateral neck area in the SPECT/CT LSG study will undergo SLNB the same day that the surgery is programmed. SNLB is based on the excision of the SLN detected by gamma-cameras in the operating room (OR). The SLN is collected and sent as a fresh sample to AP for its examination.

Meanwhile the SLNB is being processed by the pathologists, the Maxillofacial surgeons and anesthesiologists will proceed with the oncological surgery. The intervention consists of the lingual tumor removal (partial glossectomy) with imaging techniques projected in the OR as reference and ipsilateral neck dissection. Results of SLNB will be communicated into the OR so the surgery team might proceed with the additional contralateral lymphadenectomy.

Patients that showed absence of contralateral lymphatic drainage or an inconclusive result in SPECT/CT LSG will proceed with the standard surgery.

The surgical inform must be updated in the patient's clinical history and the study's data base. The later should be encoded with their encrypted ID number.

Histological analysis

The intraoperative SLNB will be processed simultaneously to the surgery. Histological examination is conducted according to protocol for sentinel lymph nodes, as elaborated in *section 2.6.3 "Imaging and histological results interpretation".*

Individuals that result in positive SLNB will be surgically intervened of the contralateral neck since we will assume presence of neck metastasis in that area. The lymphadenectomy surgical piece will be placed on an expanded polystyrene sheet with anatomical cervical references. Histological analysis will be conducted, and results will be provided in the post-operation visit.

The SLNB and the lymphadenectomy's histological examination results will be reported and uploaded into the patient's clinical history as well as to the study. These findings will be encrypted with the patient's personal study's ID.

Fourth visit: post-operative control appointment

Approximately one month following the patient's surgical procedure, a stablished follow-up appointment is scheduled. This appointment serves as an opportunity to review the histopathological findings collected during surgery, update the patient's medical records (with encrypted data into the study's database), and engage in discussions regarding any necessary further treatment options.
This is considered an ordinary visit in the health system that any post-operated oncological patient undergoes.

2-year follow-up

Oncology patients typically undergo a 5-year follow-up with routine visits and imaging studies to monitor the disease. However, our study will focus on a 2-year follow-up out of the total 5-year follow-up. Our primary objective is to detect potential neck relapses in post-operative patients, encompassing both unilateral and bilateral neck dissections. Consequently, it would confirm FN and FP results derived from our initial imaging technique (SPECT/CT LSG).

We intend to apply the standardized protocol employed by the national health system. It is commonplace that patients get scheduled every 3-4 months during the first 2-3 years of follow-up. After this time, visits will be spaced to semestral and annual appointments until the 5-year mark. If the patient has not shown any sign of the disease's relapse, they'll be considered free of disease and will be released from oncological follow-up.

In our 2-year check-up we will register a total of 8 visits (4 apt/year – 1 in every 3 months) that will include the following:

- **Visits 5-7-9-11**: Clinical interview and physical exam of oral cavity and neck. Systematic physical exam. Addressing patient and family's doubts and worries. Informing of upcoming procedures and visits and programming the next visit's imaging techniques.

- **Visits 6-8-10-12**: Utilizing a medical approach akin to previous visits, with the inclusion of imaging techniques (CT or MRI).

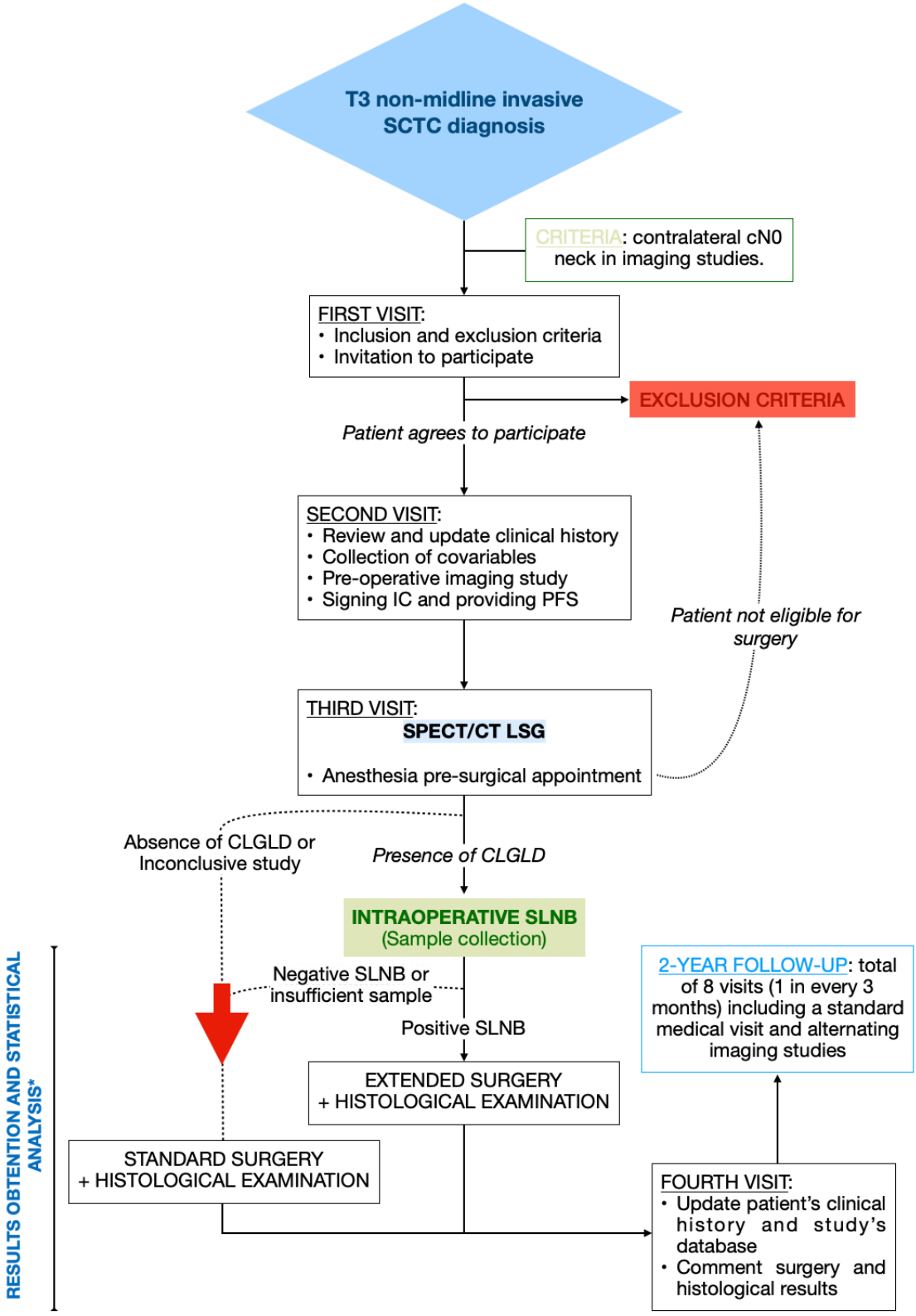


Figure 20. Patient journey overview. – *IC: informed consent; PFS: patient fact sheet; CLGLD: contralateral gammagraphic lymphatic drainage; SLNB: sentinel lymph node biopsy.* *Data collection includes the intervention and follow-up.

PERIOD (weeks)	W1	W2	W3-4	W3-4	W8	2-year follow-up							
						W20	W32	W44	W56	W68	W80	W92	W104
Visits	**First**	**Second**	**Third**	**Sample collection**	**Fourth**	**Fifth**	**Sixth**	**Seventh**	**Eighth**	**Ninth**	**Tenth**	**Eleventh**	**Twelfth**
Standard visit	✓				✓	✓	✓	✓	✓	✓	✓	✓	✓
Invitation to participate	✓												
Review and update clinical history		✓		✓	✓								
Signing IC and providing PFS		✓											
SPECT/CT LSG			✓										
Anesthesia evaluation			✓										
Surgery				✓									
SLNB				✓									
Histological examination				✓									
Post-operative results appointment					✓								
Imaging studies (MRI and CT)		✓ (pre-op)					✓		✓		✓		✓

Table 8. Resumed description of the data collection – IC: informed consent; PFS: patient fact sheet; LSG: lymphoscintigraphy; SLNB: sentinel lymph node biopsy.

7 Statistical analysis

7.1 Descriptive analysis

The histologically confirmed contralateral neck lymph node metastasis (qualitative dichotomous variable) will be summarized using proportions. This analysis will be stratified by the groups defined by presence and absence of contralateral cervical lymphatic drainage according to lymphogammagraphic studies. Entailing Sensitivity determination is the main outcome whereas Specificity and predictive values will be calculated during follow up (secondary outcome). All diagnostic validity parameters will be calculated in a bivariate inference, even though data collection will not be conducted simultaneously.

Ipsilateral lymph node stage at diagnosis is a secondary variable that can serve as an important predictor or confounder, influencing the diagnostic validity measures. Bivariate analysis will be required to assess the potential significant relationship with the study's variable (contralateral cervical lymphatic drainage) by stratifying the ipsilateral lymph node staging in groups.

Our suspicion is that tumor size can act as an influencer for the diagnostic validity measures. Therefore, it is necessary to assess the significant relation with the contralateral lymphatic drainage.

We think it is essential to describe our population by age (mean +/- SD), sex (proportion), tobacco use (proportion), alcohol consumption (proportion), educational status (proportion), N-stage at diagnosis (proportion) and tumor size (proportion).

Furthermore, stratification and subgroup modeling for the additional variables will be executed to explore their effects on diagnostic accuracy. Lastly, age will be categorized in quartiles.

7.2 Bivariate inference

This study is based on a sensitivity (SE) and specificity (SP) measures as diagnostic validity values for a test or imaging technique; lymphogammagraphy being the one of study in this project.

SE and SP serve to assess the diagnostic accuracy of a test when compared to reference criteria. In this instance, the histological examination will establish whether malignant lymph nodes are present or absent in the contralateral cervical area. These metrics enable a comparison of the test's effectiveness with others.

Sensitivity reflects a test or diagnostic tool's capacity to identify individuals affected by a disease or condition, whereas specificity assesses its ability to correctly identify individuals who are genuinely free of disease. Both statistical parameters are mathematically expressed as follows:

$$Sensitivity = \frac{True\ Positives\ (TP)}{True\ Positives\ (TP) + False\ Negatives\ (FN)}$$

$$Specificity = \frac{True\ Negatives\ (TN)}{True\ Negatives\ (TN) + False\ Positives\ (FP)}$$

Sensitivity would be calculated as the main outcome. However, for an accurate assessment of specificity, initially, negative test results will be assumed until the identification of neck relapsed patients during follow-up. The decision to use data as a proxy for SP is due to the inherent challenge of proving negative results compared to positive contralateral neck drainage, which would raise bioethical concerns related to further surgical procedures.

In this particular study, sensitivity and specificity will be calculated as ratios as outlined in Table 9:

		Histological examination for CLNM		
		Disease	Disease-Free	Total
Gammagraphic contralateral lymphatic drainage	**Positive**	TP	FP	TP+FP
	Negative	FN	TN	FN+TN
Total		TP+FN	FP+TN	n=TP+FP+TN+FN

Table 9. Computation for sensitivity and specificity.

Positive predictive value (PPV) and negative predictive value (NPV) will also be calculated to assess the diagnostic test's accuracy, in addition to specificity (SP), serving as secondary outcomes. These factors are integral to the diagnostic validity measures' examination [Table 9] and expressed as follows:

$$Positive\ predictive\ value\ (PPV) = \frac{True\ Positives\ (TP)}{True\ Positives\ (TP) + False\ Positives\ (FP)}$$

$$Negative\ predictive\ value\ (NPV) = \frac{True\ Negatives\ (TN)}{True\ Negatives\ (TN) + False\ Negatives\ (FN)}$$

The study will assess whether the proportions between both study groups exhibit statistical differences, employing McNemar's chi-square test for paired data. Additionally, stratification by covariates will be performed.

7.3 Subgroup analysis

A subgroup analysis will be conducted to evaluate possible relations with the main study's variable with secondary variables (ipsilateral N-stage at diagnosis and tumor size) and the other variables like sex, age, education status. The subgroup analysis will allow to compare the presence of contralateral cervical lymphatic drainage (study variable) across the subgroups. This will help identify if there are statistically significant differences and further suggest the need for complimentary studies regarding the results.

8 Ethical and legal considerations

This study will adhere to the medical ethics guidelines outlined in the World Medical Association's Declaration of Helsinki, titled "*Ethical Principles for Medical Research Involving Human Subjects"* (June 1964, most recently revised in 2013), as well as the foundational ethical principles set forth in 1970 by Beauchamp and Childress, with a subsequent review in 2009.

- Principle of Beneficence entails a moral duty to take actions that promote the welfare of others. Respecting beneficence, we strive to enhance survival rates through the early detection and treatment of lymph node metastasis.

- Principle of Autonomy represents the patient's free will to judge over their disease with full informed decisions. Our study will preserve this principle by providing any kind of information about the process that the patient might require. We will not condition their decision in any way, as well as facilitating a contact for them to reach out whenever they feel necessary. Autonomy will be legally represented through the informed consent (IC) [Annex VI], signed in case of the patient's agreeance to participate and supported by the patient fact sheet (PFS) [Annex VII]. The information will be presented by a Maxillofacial surgeon, emphasizing that individuals have the option to participate in the study, refuse participation, or withdraw at any time without facing any negative consequences.

- Principle of Justice ensures an equitable distribution of well-being benefits, with no discriminatory treatment based on access to health resources or factors such as socioeconomic status, ethnicity, or other distinguishing factors. Our study will not tolerate any kind of discrimination, hence preserving this maxim.

- Principle of Non-Maleficence grounds in the individual's well-being, as it involves the intention to prevent harm and mitigate the potential negative outcomes

stemming from their illness. At any indication of potential health-threatening effect, the patient will be excluded from the study.

Likewise, patients who do not exhibit any signs of occult CLNM in the LSG study or the SLNB will not undergo additional surgical intervention on the contralateral neck and will instead be included in the standard surgical group.

Data protection and confidentiality

The processing, transfer, confidentiality, and communication of personal data within this study will be governed by the following legal framework: the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons concerning the processing of personal data and the free movement of such data, repealing Directive 95/46/EC (General Data Protection Regulation); "*Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y Garantía de los derechos digitales*" (Organic Law 3/2018, of December 5, on Personal Data Protection and Guarantee of Digital Rights); and "*Real Decreto 1720/2007, de 21 de diciembre, por el que se aprueba el Reglamento de desarrollo de la Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal*" (Royal Decree 1720/2007, of December 21, approving the Regulation for the development of Organic Law 15/1999, of December 13, on Personal Data Protection).

Consequently, this study will ensure patient anonymity by assigning them a unique code in the database. Data access will be restricted to the research team exclusively. All collected data will be utilized solely for the intended purpose of this study.

Study's legal framework

This observational study legal framework will comply with the "*Real Decreto Legislativo 1/2015, de 24 de Julio, por el que se aprueba el texto refundido de la Ley de garantías y uso racional de los medicamentos y productos sanitarios*" (Legislative Royal Decree 1/2015, of July 24, approving the consolidated text of the Law on the guarantees and

rational use of medicines and healthcare products) regarding the use of imaging machinery.

Certainly, this study will adhere to the regulations outlined in "*Ley de Investigación Biomedica 14/2007, of July 3*" which governs biomedical research involving human subjects and invasive procedures.

Transparency

In the pursuit of transparency and to uphold research integrity, the following principles will be observed:

- Conflict of Interest Disclosure: All investigators are mandated to declare any potential conflict of interest.
- Complete Data and Result Publication: The research team is committed to publishing all data and results with full transparency. This includes the disclosure of unfavorable data or events.
- Study Adjustments: In the event of any modifications to the initial project plan, the research team will proactively engage with all participants to provide comprehensive information. This process will involve obtaining updated informed consent from the participants to ensure continued participation in the study."

9 Working plan

9.1 Participant centers

This study is based on a multicenter model, based on HDJT as reference and supported by HVH to recruit and intervene patients. Both are Catalan hospitals, HDJT is in the province of Girona whereas HVH is in Barcelona.

9.2 Research team

Our main research team will be composed by a multidisciplinary team including:

- Main investigator: Study Design and Planning encompass several key aspects, including the formulation of the study concept, protocol development, protocol submission to the *Comité d'Ètica en Investigació Clínica* (CEIC) for approval, participant recruitment management, oversight of the informed consent process, and supervision of data collection, analysis, interpretation, and presentation.

  The allocation of research responsibilities will also involve the main investigator overseeing coordination tasks, monitoring the study's execution, ensuring the proper implementation of the protocol, and the secure storage of data.

- Study coordinator: study monitoring and team coordination.

- Co-investigators: this team structure is equal for both HDJT and HVH.
  - **Maxillofacial Surgeons:** Responsible for performing surgery and SLNB.
  - **Nuclear Medicine Doctors**: Oversee preoperative SPECT/CT LSG and interpret results.
  - **Head and Neck Radiologists:** Key role in diagnosing and monitoring through imaging studies.
  - **Medical and Radiotherapy Oncologists:** Essential for individualized patient treatment when surgery is not feasible or requires additional adjuncts.

- **Pathological Anatomy Specialists:** Process SLNB samples and lymphadenectomy surgical pieces.
- **Anesthesia Team:** Play a crucial role in patient eligibility assessment and anesthesia management during surgery.
- **Case-management nurse**: oversee data collection during the study, coordinating the patient's journey to the committee's decision. Other responsibilities will include guiding the staff in study protocols, assisting the principal investigator in the patient's management, scheduling visits, etc.

- Data manager: Attend to data collection managing during the study. Their tasks include data processing, quality control and report writing for interim and final data analysis.

- Statistic specialist: Conduct statistical analysis of the study.

9.3 Study Stages

STAGE 0. STUDY DESIGN ***[Sep – Oct 2023]***

- **First meeting**: In the initial meeting, both the study coordinator (Dr Manel Gorina) and the principal investigator (Georgina Ducet) were present. They collectively agreed to initiate and develop the current study.

- **Protocol drafting**: The development plan and protocol memorandum were formulated between September and October 2023. This phase of the study includes bibliographical revision and meetings with specialists to review the content.

STAGE 1. ETHICAL REVISION ***[Nov 2023 – Jan 2024]***

The protocol will be submitted to the CEIC for approval, with any suggested modifications being reviewed and adjusted based on the original development plan. This will include the contracting of the insurance policy in Dec 2023.

STAGE 2. INITIAL COORDINATION ***[Jan 2024]***

The investigating team gathering will consist of a meeting composed by the study coordinator, principal investigator and co-investigators. It is sensible to determine a representative of the co-investigators (both in HDJT and HVH), preferably a maxillofacial surgeon since this team will be responsible of the foremost part of the intervention. The meeting aims to assign roles, establish a work plan and system, and develop a program of tasks that will be assigned to each specialist.

Additionally, the co-investigators' leader of HVH will attend to a workshop regarding the project's protocol to standardize the procedures.

STAGE 3. RECRUITMENT AND DATA COLLECTION ***[Feb 2024 – Jan 2028]***

- **Recruitment and intervention (Feb 2024 – Jan 2026)**: We will employ a consecutive non-probabilistic sampling method to enroll patients over a 2-year period. Eligibility for the study requires that patients meet all inclusion criteria, satisfy none of the exclusion criteria, and provide informed consent. Following this, patients will undergo LSG. Based on the LSG results, patients will be divided into two groups. Group 1 (absence of CLNLD) will proceed directly to protocol surgery without SLNB, while Group 2 (presence of CLNLD) will undergo SLNB. Subsequent actions for Group 2, including the potential need for additional contralateral neck surgery, will be determined based on the SLNB results.

- **Data collection and follow-up (Feb 2024 – Jan 2028)**: generally, it will be carried out simultaneously to recruitment (Feb 2024 – Jan 2026). Yet, it will require an

extra 2 years of follow-up after the surgical intervention to collect the necessary data to complete the second outcome (Feb 2026 – Jan 2028). The follow-up visits will include conducting systematic clinical evaluations, administering blood tests, and periodically perform imaging studies. These assessments will be complemented by a combination of in-person and remote telemedicine visits. The main investigator, study coordinator and head of co-investigators will conduct this part. The data manager will register the data derived from this phase.

In line with studies such as *Gónzalez-García et al*, post-operative lymph node neck recurrence was 11,4 months (56). Consequentially, we suggest a 2-year close observation to detect cervical relapse.

- **Quality control (Feb 2024 – Jan 2028)**: audits periodically the research from recruitment and data collection. Verifies that clinical history and data retrieval is the same.

In this phase, control meetings will be arranged among the principal investigator, study coordinator, co-investigator lead, and data manager to ensure that the study is progressing as intended.

STAGE 4. STATISTICAL ANALYSIS ***[Feb – May 2028]***

- **Statistical analysis (Feb – Mar 2028):** The statistician will ensure the corresponding analyses with all the information collected once stage 3 is completed.

- **Interpretation of results (Ap – May 2028):** The results will be analyzed, and conclusions will be formulated. If necessary, meetings might be arranged to address this matter for its correct interpretation correlated to the clinical state.

STAGE 5. RESULTS PUBLICATION ***[Jun – Sep 2028]***

- **Paper Preparation**: We will compile a paper to present the study's results and conclusions.

- **Congress Presentation**: We plan to participate in a national congress (*Sociedad Española de Cirugía Oral y Maxilofacial y Cabeza y Cuello*) to share our findings throughout a lecture of the study.

- **Publication**: This phase consists of the paper's submission to various scientific journals for publication. Our main target journal is the Spanish Society of Oral and Maxillofacial Surgery.

The primary responsibility for these tasks will lie with the principal investigator and study coordinator.

STAGE	TASK	STAFF	PERIOD													
			2023				2024		2025	2026		2027	2028			
			Sep	Oct	Nov	Dec	Jan	Feb-Dec	Jan-Dec	Jan	Feb-Dec	Jan-Dec	Jan	Feb-Mar	Ap-May	Jun-Sep
0. Study design	First meeting	Study coordinator Principal investigator														
	Protocol draft	Principal investigator														
1. Ethical revision	Presentation to CEIC and Insurance contracting	Study coordinator Principal investigator CEIC board														
2. Initial Coordination	Investigating team gathering	Study coordinator Principal investigator Co-investigators														
	Team workshop	Study coordinator Principal investigator Head of HDJT and HVH co-investigators														
3. Recruitment and data collection	Recruitment	Investigators Co-investigators														
	Data record	Investigators Co-investigators Data manager														
	Quality control	Data and quality control manager														
4. Statistical Analysis	Statistical analysis	Statistician														
	Data interpretation	Coordinator Principal investigator														
5. Results publication	Publication and dissemination	Coordinator Principal investigator														

Table 10. Chronogram – CEIC: Comité d'Ètica d'Investigació Clínica; ;HDJT: Hospital Dr Josep trueta; HVH: Hospital Vall d'Hebrón.

10 Budget

STAFF

The budget does not include the cost of the investigating team which is formed by the main investigator and co-investigator, study coordinator and collaborators. This team counts with Maxillofacial surgeons, Nuclear Medicine doctors, Radiologists, Pathologists, Anesthesiologists and medical and radiological oncologists. All medical professionals are part of the national health system.

Subcontracted services are accounted in the expenses of the project's realization.
We will enlist the services of an independent statistician to conduct the statistical analysis on the gathered data. The estimated hourly rate for this professional will be 30€, resulting in a total expense of 1,350€ for 45 hours of work.

Regarding data collection, creation of database and quality control, we will employ a data manager. The expected hourly wage for this role will be 45€, resulting in a total expenditure of 2,250€ for 50 hours of work.

INSURANCE

Should the CEIC perceive this study as invasive, obtaining liability insurance will become necessary to address any potential adverse events stemming from the study. The estimated expense is approximately 35,000€.

MATERIALS AND EXECUTION

The execution for this study requires three main points: the SPECT/CT LSG combined with SLNB and posterior complimentary contralateral neck dissection.

We budgeted an estimated price of 800€/patient for the SPECT/CT LSG study which includes the radiotracer and machinery. We plan on executing it on all our sample (118 patients) which results in a total of 94,400€.

Technically, only patients that show presence of contralateral neck drainage in the previous technique would undergo SLNB. However, since we do not possess that information at the given time, we anticipated SLNB for 80% of our sample (94 patients). The cost for this technique and its histological examination rounds up to 450€/patient (total: 42,300€).

All patients participating in the study will undergo surgical intervention as needed, which includes partial glossectomy and ipsilateral neck dissection. However, contralateral lymphadenectomy will be conducted solely for those who receive a positive SLNB result. To simplify the approach, we assumed that all 80% of the sample (94 patients) who underwent SLNB would test positive, thus justifying the continuation of surgery with the additional contralateral lymphadenectomy. The supplementary cost of the contralateral cervical lymphadenectomy to the standard procedure (part of the protocol – not included in budget) is estimated as 200€/patient (total: 18,800€).

Printing costs are mainly related to the informed consent authorization and the patient fact sheet that will be handed to all patients at the beginning of the study. Printing expenses round to 0.03€/page (total: 21.24€).

Even though the team is thoroughly experienced with the SNLB technique, as well as with the SPECT/CT LSG, since its considered standard practice for early staged SCTC, we suggest a training session to unify the project's protocol. This workshop will be held in *Hospital Dr Josep Trueta* and will require of the project's team leader of *Hospital Vall d'Hebrón.* The estimated expense for a morning workshop including travelling and diet accounts for 60€.

Other expenses not included in the budget include medical appointments regarding diagnosis, treatment decision, imaging and blood tests (CT, MRI, general blood work as defined by protocol), individualized treatment options – surgery (partial glossectomy +/- ipsilateral cervical lymphadenectomy), radiotherapy, chemotherapy -, follow up visits with routine imaging techniques. The cited clinical procedures are included throughout the normal diagnostic and therapeutic process covered by the national health system.

PUBLICATION FEES AND DISSEMINATION

The paper resulting from this protocol will be published in an Open Access journal to enhance visibility and accessibility. While our preferred target journal is likely to be the Spanish Society of Oral and Maxillofacial Surgery Journal (which typically covers publication fees), we have budgeted an estimated cost of 2,000€ to account for peer review, editing, and other derived expenses.

Attendance to a relevant national congress as *Sociedad Española de Cirugía Oral y Maxilofacial y de Cabeza y Cuello (SECOM CyC)* is accounted for the main investigator and the study coordinator. The primary goal is to share the research findings with the H&N scientific community. The cost of 800€ per attendee covers access to the congress, as well as travel expenses, meals, and accommodation. The total projected cost for two attendees amounts to 1,600€.

Item	Quantity	Cost	Subtotal
STAFF			
Statistician	45h	30€/h	1,350€
Data management and quality control	50h	45€/h	2,250€
INSURANCE POLICY			
Trial policy	1	35,000€	35,000€
MATERIALS AND EXECUTION			
SPECT/CT LSG	118	800€/patient	94,400€
SLNB (including AP)	94	600€/patient	42,300€

Additional contralateral lymphadenectomy to standardized surgery	94	200€/patient	18,800€
Team training	1 attendee	60€/person	60€
Printing costs	708 pages	0.03€/page	21.24€
PUBLICATION FEES AND DISSEMINATION			
Publication fees	1	2000€/publication	2,000€
Attendance to *Sociedad Española de Cirugía Oral y Maxilofacial y de Cabeza y Cuello*	1 national congress 2 attendees	800€/attendee	1,600€
			TOTAL: 197,781.24 €

Table 11. Budget overview.

11 Limitations of the study

This study faces multiple limitations:

- **Extrapolation of Results**: One of the key limitations of this study is the potential for results to be extrapolated to a broader population. This is due to the study's relatively restrictive inclusion/exclusion criteria. The inclusion of a narrow range of patient profiles can limit the generalizability of the findings to the general population.

- **Bias in Observational Study**: Although this study is classified as observational, meaning it primarily focuses on collecting, presenting and summarizing data, it's essential to acknowledge that there is always the potential for bias in data collection and analysis. These data collection and analysis biases are planned to be controlled by the data manager and periodical quality control audits to minimize their effects.

  Another bias that may be present in this type of studies is measurement bias between the participant centers that will be eradicated by a technique protocol-unifying workshop and periodical meetings between the centers' referents of the study. Regarding confounding bias, we have not identified any confounders since we cannot establish associations between our study's variables and diagnostic validity measures. However, we intend to address potential confounding by applying subgroup modeling to the remaining variables that we believe may influence our diagnostic validity measures.

- **Proxy Data Use**: Perhaps the most significant limitation is the reliance on proxy data. The study makes assumptions about negative results in SPECT/CT LSG and SLNB and awaits confirmation during follow-up by observing neck relapse. This introduces an element of uncertainty, as relying solely on proxy data may not capture the true extent of the phenomenon being studied. The study's initial conclusions may be limited by the accuracy and reliability of these proxy indicators.

Nevertheless, it is not free of its share of strengths regarding:

- **Wide Sample**: One notable strength of this study is the inclusion of a substantial sample size, involving 118 patients. This large and diverse sample contributes to the robustness of the findings and offers the potential for insights that may not have been apparent in smaller studies. The study's breadth sets it apart from previous research, as similar studies have not previously included such a wide range of patients.

- **Originality and Technological Innovation**: The study demonstrates originality by applying new technologies to the current situation of tongue cancer within a specific environment. This innovative approach contributes to the advancement of knowledge in the field and may provide valuable insights that can inform clinical practice. The incorporation of novel technologies is a strength that distinguishes this research from conventional studies and opens opportunities for groundbreaking discoveries.

In conclusion, while this study has certain limitations, such as the potential for extrapolation, the presence of biases, and the use of proxy data, it also offers substantial strengths in terms of its extensive sample size and its innovative use of technology. Researchers should be aware of these limitations while appreciating the unique contributions and potential implications of the study's findings. The strengths and weaknesses should be considered when interpreting and applying the results to clinical practice.

While our study will play a crucial role in establishing the diagnostic accuracy of sentinel node study techniques, we recommend conducting subsequent research to compare them with conventional imaging methods. Additionally, further investigation into the implications of bilateral lymphadenectomy and radiotherapy, guided by the outcomes of the sentinel node study, is warranted.

12 Feasibility

This multicenter study will take place at 2 hospitals based in Catalonia (Spain), *Hospital Universitari Doctor Josep Trueta* and *Hospital Vall d'Hebrón,* both of which possess the requisite resources and suitable facilities for conducting the study due to its third-level assistance. Periodical meetings will be scheduled to guarantee proper communication between centers.

Over a two-year period, a sample of 118 patients will be recruited in a multicentric model, ensuring an adequate number of participants without exceeding the specified timeframe. Individuals will be enlisted from the Oral and Maxillofacial Department of the respective hospitals and will have to be included in the head and neck tumors' multidisciplinary committee.

SPECT/CT lymphogammagraphy machinery, as well as all the necessary devices for the sentinel node biopsy, are already available in both centers. In oral cancer, it is already a standard of care in early staged tumors, so we do not expect incidences in requesting these techniques or the radiotracer for our study.

Patients will undergo the sentinel node study a day before or the same day to surgery. The following visits are indicated as follow-up observation after surgery and for disease evolution. Except from the sentinel node study that we aim to validate with this study, all therapeutic approach will be conducted according to clinical guidelines used in both hospitals.

The budget is reasonable, as all participating hospitals already have the necessary equipment for the intervention. Aside from hiring a statistician and a data manager, no additional personnel will be required.

13 References

1. Globocan. Lip and oral cancer factsheet [Internet]. WHO; Available from: https://gco.iarc.fr/today/data/factsheets/cancers/1-Lip-oral-cavity-fact-sheet.pdf
2. Gonzalez M, Riera March A. Tongue Cancer. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 Jul 15]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK562324/
3. Ng JH, Iyer NG, Tan MH, Edgren G. Changing epidemiology of oral squamous cell carcinoma of the tongue: A global study. Head Neck. 2017 Feb;39(2):297–304.
4. Porta-Vázquez M, López-Cedrún JL, Fernández-Sanromán J, García-Mato E, Diz-Dios P, Diniz-Freitas M. Estimating the direct costs of oral cancer in Spain: a retrospective hospital data analysis. Med Oral Patol Oral Cir Bucal [Internet]. 2023 Sep [cited 2023 Sep 29];28(5):e425–32. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10499339/
5. Som PM, Curtin HD. Head and Neck Imaging: Expert Consult- Online and Print. Elsevier Health Sciences; 2011. 1865–934 p.
6. Ram H, Sarkar J, Kumar H, Konwar R, Bhatt MLB, Mohammad S. Oral Cancer: Risk Factors and Molecular Pathogenesis. J Maxillofac Oral Surg [Internet]. 2011 Jun [cited 2023 Sep 19];10(2):132–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3177522/
7. Kato MG, Baek CH, Chaturvedi P, Gallagher R, Kowalski LP, Leemans CR, et al. Update on oral and oropharyngeal cancer staging – International perspectives. World J Otorhinolaryngol - Head Neck Surg [Internet]. 2020 Mar 6 [cited 2023 Jul 16];6(1):66–75. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7221211/
8. Honorato J, Rebelo MS, Dias FL, Camisasca DR, Faria PA, Azevedo e Silva G, et al. Gender differences in prognostic factors for oral cancer. Int J Oral Maxillofac Surg [Internet]. 2015 Oct 1 [cited 2023 Sep 19];44(10):1205–11. Available from: https://www.sciencedirect.com/science/article/pii/S0901502715001757
9. Azimi S, Ghorbani Z, Ghasemi E, Tennant M, Kruger E. Does socioeconomic status influence oral cancer awareness? The role of public education. East Mediterr Health J Rev Sante Mediterr Orient Al-Majallah Al-Sihhiyah Li-Sharq Al-Mutawassit. 2020 Dec 9;26(12):1510–7.
10. Hussein AA, Forouzanfar T, Bloemena E, de Visscher J, Brakenhoff RH, Leemans CR, et al. A review of the most promising biomarkers for early diagnosis and prognosis prediction of tongue squamous cell carcinoma. Br J Cancer [Internet]. 2018 Sep 11 [cited 2023 Aug 23];119(6):724–36. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6173763/
11. Alcohol Beverage Consumption [Internet]. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service; 2021. (National Toxicology Program N, editor. Report on Carcinogens, Fifteenth edition). Available from: https://ntp.niehs.nih.gov/go/roc15
12. Luo CW, Roan CH, Liu CJ. Human papillomaviruses in oral squamous cell carcinoma and pre-cancerous lesions detected by PCR-based gene-chip array. Int J Oral Maxillofac Surg [Internet]. 2007 Feb 1 [cited 2023 Sep 24];36(2):153–8. Available from: https://www.sciencedirect.com/science/article/pii/S0901502706003766
13. Yang SW, Lee YS, Chen TA, Wu CJ, Tsai CN. Human papillomavirus in oral leukoplakia is no prognostic indicator of malignant transformation. Cancer Epidemiol

[Internet]. 2009 Aug 1 [cited 2023 Sep 24];33(2):118–22. Available from: https://www.sciencedirect.com/science/article/pii/S1877782109000538
14. Atula T, Grénman R, Klemi P, Syrjänen S. Human papillomavirus, Epstein-Barr virus, human herpesvirus 8 and human cytomegalovirus involvement in salivary gland tumours. Oral Oncol. 1998 Sep;34(5):391–5.
15. Al Moustafa AE, Chen D, Ghabreau L, Akil N. Association between human papillomavirus and Epstein-Barr virus infections in human oral carcinogenesis. Med Hypotheses. 2009 Aug;73(2):184–6.
16. Vadovics M, Ho J, Igaz N, Alföldi R, Rakk D, Veres É, et al. Candida albicans Enhances the Progression of Oral Squamous Cell Carcinoma In Vitro and In Vivo. mBio. 2021 Feb 22;13(1):e0314421.
17. Drake RL, Vogl AW, Mitchell AW. Anatomy of the tongue. In: Gray's Anatomy for Students [Internet]. Fourth edition. Philadelphia: Elsevier Health Sciences; [cited 2023 Jul 15]. p. 1084–90. Available from: https://bookshelf.health.elsevier.com/reader/books/9780323508520/epubcfi/6/120[%3Bvnd.vst.idref%3Did_chp00008_s6]!/4/2/2/2[s0170]/2/2[s3065]/2[st3025]/1:9[avi%2Cty]
18. Roi A, Roi CI, Andreescu NI, Riviş M, Badea ID, Meszaros N, et al. Oral cancer histopathological subtypes in association with risk factors: a 5-year retrospective study. Rom J Morphol Embryol [Internet]. 2020 [cited 2023 Sep 24];61(4):1213–20. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8343614/
19. Speight PM, Farthing PM. The pathology of oral cancer. Br Dent J [Internet]. 2018 Nov [cited 2023 Sep 19];225(9):841–7. Available from: https://www.nature.com/articles/sj.bdj.2018.926
20. Ciucâ FI, Marasescu PC, Matei M, Florescu AM, Margariteascu C, Petrescu SMS, et al. Epidemiological and Histopathological Aspects of Tongue Squamous Cell Carcinomas-Retrospective Study. Curr Health Sci J [Internet]. 2018 [cited 2023 Sep 20];44(3):211–24. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6311229/
21. Hsu PJ, Yan K, Shi H, Izumchenko E, Agrawal N. Molecular Biology of Oral Cavity Squamous Cell Carcinoma. Oral Oncol [Internet]. 2020 Mar [cited 2023 Sep 25];102:104552. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7659372/
22. Sharma M, Sah P, Sharma SS, Radhakrishnan R. Molecular changes in invasive front of oral cancer. J Oral Maxillofac Pathol JOMFP [Internet]. 2013 [cited 2023 Sep 29];17(2):240–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3830234/
23. Sharma D, Koshy G, Grover S, Sharma B. Sentinel Lymph Node Biopsy. Sultan Qaboos Univ Med J [Internet]. 2017 Feb [cited 2023 Oct 19];17(1):e3–10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5380418/
24. Sinha GM, Hegde V. Detection of micro-metastasis using cytokeratins (AE1/AE3) in haematoxylin & eosin-stained N0 lymph nodes of oral squamous cell carcinoma. Indian J Med Res [Internet]. 2023 Apr [cited 2023 Oct 23];157(4):316–21. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10438399/
25. Hema KN, Smitha T, Sheethal HS, Mirnalini SA. Epigenetics in oral squamous cell carcinoma. J Oral Maxillofac Pathol JOMFP [Internet]. 2017 [cited 2023 Sep 25];21(2):252–9. Available from:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5596676/
26. Bagan J, Sarrion G, Jimenez Y. Oral cancer: Clinical features. Oral Oncol [Internet]. 2010 Jun 1 [cited 2023 Sep 25];46(6):414–7. Available from: https://www.sciencedirect.com/science/article/pii/S1368837510000989
27. Ong CK, Chong VFH. Imaging of tongue carcinoma. Cancer Imaging [Internet]. 2006 Dec 20 [cited 2023 Oct 16];6(1):186–93. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1766559/
28. Sigal R, Zagdanski AM, Schwaab G, Bosq J, Auperin A, Laplanche A, et al. CT and MR imaging of squamous cell carcinoma of the tongue and floor of the mouth. Radiogr Rev Publ Radiol Soc N Am Inc. 1996 Jul;16(4):787–810.
29. Shah JP, Montero PH. New AJCC/UICC staging system for head and neck, and thyroid cancer. Rev Médica Clínica Las Condes [Internet]. 2018 Jul 1 [cited 2023 Sep 29];29(4):397–404. Available from: https://www.elsevier.es/es-revista-revista-medica-clinica-las-condes-202-articulo-new-ajcc-uicc-staging-system-for-S0716864018300919
30. Akamatsu M, Makino T, Morita S, Noda Y, Kariya S, Onoda T, et al. Midline involvement and perineural invasion predict contralateral neck metastasis that affects overall and disease-free survival in locally advanced oral tongue squamous cell carcinoma. Front Oncol [Internet]. 2022 Oct 26 [cited 2023 Oct 3];12:1010252. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9644196/
31. Lloyd S, Yu JB, Wilson LD, Judson BL, Decker RH. The Prognostic Importance of Midline Involvement in Oral Tongue Cancer. Am J Clin Oncol [Internet]. 2012 Oct [cited 2023 Oct 19];35(5):468–73. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3755371/
32. den Toom IJ, Janssen LM, van Es RJJ, Karagozoglu KH, de Keizer B, van Weert S, et al. Depth of invasion in patients with early stage oral cancer staged by sentinel node biopsy. Head Neck [Internet]. 2019 [cited 2023 Oct 19];41(7):2100–6. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/hed.25665
33. Berdugo J, Thompson LDR, Purgina B, Sturgis CD, Tuluc M, Seethala R, et al. Measuring Depth of Invasion in Early Squamous Cell Carcinoma of the Oral Tongue: Positive Deep Margin, Extratumoral Perineural Invasion, and Other Challenges. Head Neck Pathol [Internet]. 2018 Apr 26 [cited 2023 Oct 19];13(2):154–61. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6514023/
34. Pfister DG, Spencer S, Adkins D, Birkeland AC, Brizel DM, Busse PM, et al. NCCN Guidelines for Head & Neck Cancers. National Comprehensive Cancer Network (NCCN); 2023 May.
35. Stephan K. H, Sandro J. S. SPECT/CT for Lymphatic Mapping of Sentinel Nodes in Early Squamous Cell Carcinoma of the Oral Cavity and Oropharynx. Int J Mol Imaging [Internet]. 2011 [cited 2023 Oct 18];2011:106068. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3065910/
36. den Toom IJ, Mahieu R, van Rooij R, van Es RJJ, Hobbelink MGG, Krijger GC, et al. Sentinel lymph node detection in oral cancer: a within-patient comparison between [99mTc]Tc-tilmanocept and [99mTc]Tc-nanocolloid. Eur J Nucl Med Mol Imaging [Internet]. 2021 [cited 2023 Oct 19];48(3):851–8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8036184/
37. Giammarile F, Schilling C, Gnanasegaran G, Bal C, Oyen WJG, Rubello D, et al. The EANM practical guidelines for sentinel lymph node localisation in oral cavity squamous cell carcinoma. Eur J Nucl Med Mol Imaging. 2019 Mar;46(3):623–37.

38. Can Sentinel Lymph Node Biopsy Predict Various Levels of Echelon Nodes in Oral Cancers? - PubMed [Internet]. [cited 2023 Oct 29]. Available from: https://pubmed.ncbi.nlm.nih.gov/32256831/
39. Alkureishi LWT, Burak Z, Alvarez JA, Ballinger J, Bilde A, Britten AJ, et al. Joint Practice Guidelines for Radionuclide Lymphoscintigraphy for Sentinel Node Localization in Oral/Oropharyngeal Squamous Cell Carcinoma. Ann Surg Oncol [Internet]. 2009 [cited 2023 Oct 18];16(11):3190–210. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2766455/
40. Villarreal P, García-Rozado A, Cebrián-Carretero J. Biopsia de los ganglios centinelas cervicales en el carcinoma de células escamosas oral y orofaríngeo. SECOM; 2014 Jan p. 523–36. (Protocolos clínicos de la Sociedad Española de Cirugía Oral y Maxilofacial). Report No.: Chapter 38.
41. Sørensen JA, Bakholdt VT, Grupe P, Thomsen JB. Sentinel lymph node biopsy for oral cancer. J Vis Surg [Internet]. 2018 Aug 2 [cited 2023 Oct 19];4(0). Available from: https://jovs.amegroups.org/article/view/20624
42. Yandrapalli S, Puckett Y. SPECT Imaging. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 Oct 19]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK564426/
43. Choi KY, Park SC, Kim JH, Lee DJ. The occult nodal metastasis rate of early tongue cancer (T1–T2). Medicine (Baltimore) [Internet]. 2021 Jan 22 [cited 2023 Aug 3];100(3):e24327. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7837872/
44. Pentenero M, Cistaro A, Brusa M, Ferraris MM, Pezzuto C, Carnino R, et al. Accuracy of 18F-FDG-PET/CT for staging of oral squamous cell carcinoma. Head Neck [Internet]. 2008 [cited 2023 Jun 8];30(11):1488–96. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/hed.20906
45. Kim SJ, Pak K, Kim K. Diagnostic accuracy of F-18 FDG PET or PET/CT for detection of lymph node metastasis in clinically node negative head and neck cancer patients; A systematic review and meta-analysis. Am J Otolaryngol [Internet]. 2019 Mar 1 [cited 2023 Sep 25];40(2):297–305. Available from: https://www.sciencedirect.com/science/article/pii/S0196070918308202
46. Liu M, Wang SJ, Yang X, Peng H. Diagnostic Efficacy of Sentinel Lymph Node Biopsy in Early Oral Squamous Cell Carcinoma: A Meta-Analysis of 66 Studies. PloS One. 2017;12(1):e0170322.
47. Agrawal A, Civantos FJ, Brumund KT, Chepeha DB, Hall NC, Carroll WR, et al. [(99m)Tc]Tilmanocept Accurately Detects Sentinel Lymph Nodes and Predicts Node Pathology Status in Patients with Oral Squamous Cell Carcinoma of the Head and Neck: Results of a Phase III Multi-institutional Trial. Ann Surg Oncol. 2015 Oct;22(11):3708–15.
48. Govers TM, Hannink G, Merkx MAW, Takes RP, Rovers MM. Sentinel node biopsy for squamous cell carcinoma of the oral cavity and oropharynx: a diagnostic meta-analysis. Oral Oncol. 2013 Aug;49(8):726–32.
49. Alvarez J, Bidaguren A, McGurk M, Diaz-Basterra G, Brunsó J, Andikoetxea B, et al. Sentinel node biopsy in relation to survival in floor of the mouth carcinoma. Int J Oral Maxillofac Surg. 2014 Mar;43(3):269–73.
50. Schilling C, Gnansegaran G, Thavaraj S, McGurk M. Intraoperative sentinel node imaging versus SPECT/CT in oral cancer - A blinded comparison. Eur J Surg Oncol J Eur Soc Surg Oncol Br Assoc Surg Oncol. 2018 Dec;44(12):1901–7.

51. Singh P, Kaul P, Singhal T, Kumar A, Garg PK, Narayan ML. Role of Sentinel Lymph Node Drainage Mapping for Localization of Contralateral Lymph Node Metastasis in Locally Advanced Oral Squamous Cell Carcinoma – A Prospective Pilot Study. Indian J Nucl Med IJNM Off J Soc Nucl Med India [Internet]. 2023 [cited 2023 Oct 2];38(2):125–33. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10348499/
52. Schilling C, Stoeckli SJ, Vigili MG, de Bree R, Lai SY, Alvarez J, et al. Surgical consensus guidelines on sentinel node biopsy (SNB) in patients with oral cancer. Head Neck [Internet]. 2019 [cited 2023 Oct 2];41(8):2655–64. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/hed.25739
53. Schilling C, Stoeckli SJ, Haerle SK, Broglie MA, Huber GF, Sorensen JA, et al. Sentinel European Node Trial (SENT): 3-year results of sentinel node biopsy in oral cancer. Eur J Cancer Oxf Engl 1990. 2015 Dec;51(18):2777–84.
54. Habib MdA, Rahman QB, Hossain S, Imon AA, Kundu GC. Effectiveness of Preoperative Lymphoscintigraphy for the Detection of Cervical Lymph Node Metastasis in Patients with Oral Squamous Cell Carcinoma. Ann Maxillofac Surg [Internet]. 2017 [cited 2023 Oct 17];7(1):30–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5502512/
55. Killelea BK, Long JB, Dang W, Mougalian SS, Evans SB, Gross CP, et al. Associations Between Sentinel Lymph Node Biopsy and Complications for Patients with Ductal Carcinoma In Situ. Ann Surg Oncol [Internet]. 2018 [cited 2023 Oct 19];25(6):1521–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5928184/
56. González-García R, Naval-Gías L, Sastre-Pérez J, Rodríguez-Campo FJ, Muñoz-Guerra MF, Usandizaga JLGD, et al. Contralateral lymph neck node metastasis of primary squamous cell carcinoma of the tongue: a retrospective analytic study of 203 patients. Int J Oral Maxillofac Surg [Internet]. 2007 Jun 1 [cited 2023 Aug 3];36(6):507–13. Available from: https://www.ijoms.com/article/S0901-5027(07)00056-2/fulltext
57. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid Off J Am Thyroid Assoc. 2009 Nov;19(11):1167–214.
58. Shah JP, Candela FC, Poddar AK. The patterns of cervical lymph node metastases from squamous carcinoma of the oral cavity. Cancer [Internet]. 1990 Jul 1;66(1):109–13. Available from: https://pubmed.ncbi.nlm.nih.gov/2354399/
59. Farmer RW, McCall L, Civantos FJ, Myers JN, Yarbrough WG, Murphy B, et al. Lymphatic Drainage Patterns in Oral Squamous Cell Carcinoma: Findings of the ACOSOG Z0360 (Alliance) Study. Otolaryngol--Head Neck Surg Off J Am Acad Otolaryngol-Head Neck Surg [Internet]. 2015 Apr [cited 2023 Jun 8];152(4):673–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4399646/
60. Thoenissen P, Heselich A, Deeg S, Al-Maawi S, Tanneberger A, Sader R, et al. Extent of Neck Dissection and Cervical Lymph Node Involvement in Oral Squamous Cell Carcinoma. Front Oncol [Internet]. 2022 May 24 [cited 2023 Jun 8];12:812864. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9172998/
61. Zanoni DK, Patel SG, Shah JP. Changes in the 8th Edition of the American Joint Committee on Cancer (AJCC) Staging of Head and Neck Cancer: Rationale and Implications. Curr Oncol Rep [Internet]. 2019 Apr 17 [cited 2023 Oct 16];21(6):52. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6528815/

62. International Standard Classification of Education (ISCED) [Internet]. [cited 2023 Oct 19]. Available from: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=International_Standard_Classification_of_Education_(ISCED)

14 Annexes

14.1 Annex I: Cervical lymph node levels

Cervical lymph nodes are generally classified by compartments that separate into six levels (I-VI) and sublevels (A-B). Each level is comprised by key anatomical structures.

LEVEL I: submental and submandibular.

- **IA - submental nodes**: anteromedial region between anterior belly of the digastric muscles.
- **IB - submandibular nodes**: posterolateral to the digastric muscles' anterior belly.

LEVEL II: upper internal jugular or deep cervical chain.

- **IIA – jugulodigastric nodes**: anterior to the posterior edge of the internal jugular vein.
- **IIB**: posterior to the edge of the internal jugular vein.

LEVEL III: mid-jugular or middle deep cervical chain.

LEVEL IV: lower internal jugular deep chain. Includes medial supraclavicular nodes (i.e. Virchow node).

LEVEL V: posterior triangle.

- **VA – upper posterior triangle**: the superior border of the cricoid cartilage (posterior to II and III), encompassing the spinal accessory nodes.
- **VB – lower posterior triangle**: the inferior border of the cricoid cartilage (located behind level IV), including the lateral supraclavicular nodes.

LEVEL VI: central or anterior.

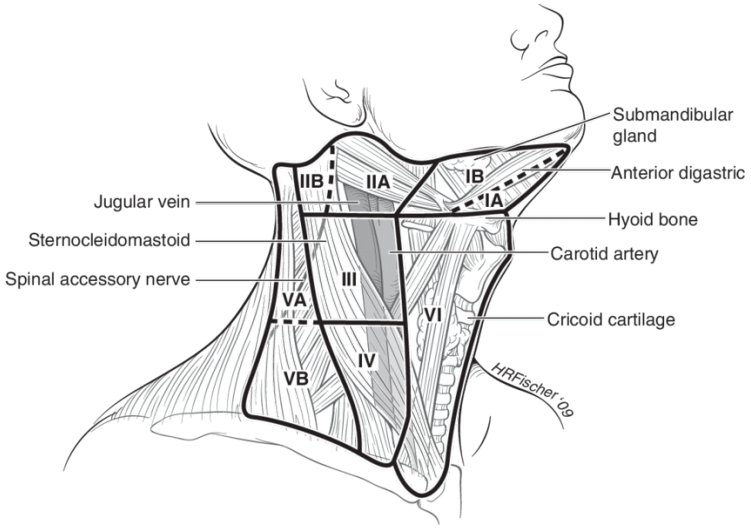


Figure 21. Lymph node compartments separated by levels and sublevels. Extracted from Cooper, D.S et al (57).

14.2 Annex II: Lymph node distribution of oral cancer

The lymphatic system related to the oral cavity drains into the deep cervical lymph node chain found along the internal jugular vein.

The oral tongue (anterior two-thirds) drains directly into previously mentioned lymph node chain trespassing through the mylohyoid muscle and submental (inferior to mylohyoid muscle and between digastric muscles) and submandibular (below oral cavity floor) nodes (17).

Studies have concluded that oral cancer frequently disseminates through lymphatic networks into levels I and II [Table 12].

Studies x Level	I	II	III	IV	V
Shah et al	19.8%	10.9%	8.9%	3.1%	0.5%
Farmer RW et al	10.9%	15.9%	7.2%	1.4%	0 %
Thoenissen et al	39.47%	38.95%	10.53%	11.05%	-

Table 12. Comparison of different studies in the distribution of ganglion metastases in oral cavity cancer. Adapted from Shah et al (58), Farmer RW et al (59) and Thoenissen et al (60).

14.3 Annex III: AJCC TNM staging of tongue cancer (8th ed)

According to the latest TNM edition (8th edition) accorded by the American Joint Committee on Cancer (AJCC), TC is staged as follows:

TNM staging system for tongue cancer			
Tumor (T)	Tx	Primary tumor cannot be assessed.	
	T0	No evidence of a primary tumor.	
	Tis	Carcinoma in situ.	
	T1	Tumor is 2 cm or smaller in greatest dimension. DOI ≤ 5mm.	
	T2	Tumor is larger than 2 cm but not larger than 4 cm. DOI >5mm but ≤10mm.	
	T3	Tumor is larger than 4 cm. DOI > 10mm.	
	T4	Tumor has deep invasion.	
		T4a	Deep invasion of tongue muscles.
		T4b	Invasion to adjacent structures (i.e., oral floor, mandible, facial skin…)
Lymph node involvement (N)	Nx	Regional lymph nodes cannot be assessed.	
	N0	No regional adenopathic involvement.	
	N1	Single ipsilateral lymph node 3cm or smaller in greatest dimension.	
	N2	Ipsilateral or contralateral lymph node(s), all smaller than 6cm.	
		N2a	Single ipsilateral lymph node between 3-6cm.
		N2b	Multiple ipsilateral lymph nodes, each 6cm or smaller.
		N2c	Contralateral or bilateral lymph nodes, each 6cm or smaller.
	N3	Any lymph node measuring 6cm or larger.	
		N3a	Single lymph node 6cm or greater.
		N3b	Multiple ipsilateral or contralateral lymph node, one of them measuring 6cm or larger.
	M0	No distant metastasis.	

Metastasis (M)	M1	Presence of distant metastasis.

Table 13. TNM staging for lingual cancer. Based off AJCC 8th ed (29).

CLINICAL STAGING:

TNM provides a stage grouping that eases patient prognosis and treatment planning that ranges between stage 0 to IV [Table 14].

STAGE 0		Tis	N0	M0
STAGE I		T1	N0	M0
STAGE II		T2	N0	M0
STAGE III		T1	NI	M0
		T2		
		T3	N0	M0
			N1	
STAGE IV	**IVA**	T1	N2	M0
		T2		
		T3		
		T4a	N0	M0
			N1	
			N2	
	IVB	Any T	N3	M0
		T4b	Any N	M0
	IVC	Any T	Any N	M1

Table 14. Clinical staging by TNM grouping. Based off AJCC 8th ed (29,61).

14.4 Annex IV: NCCN guidelines for SCTC treatment

EARLY-STAGE TREATMENT ALGORITHM

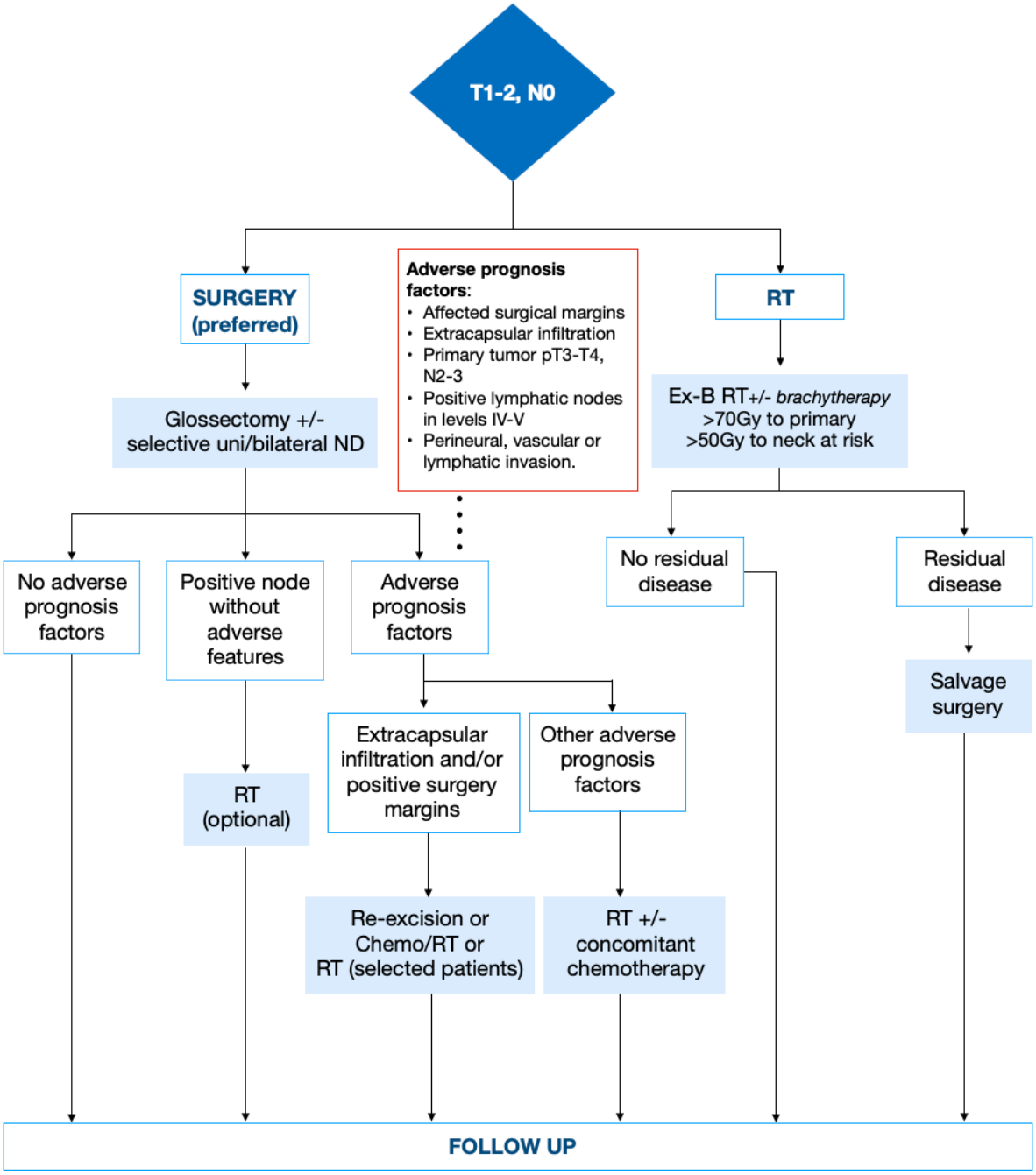


Figure 22. Surgical and adjuvant treatment of early stages of SCTC. – ND: neck dissection; RT: radiotherapy; Ex-B: external-beam. *Adapted from NCCN.*

ADVANCED-STAGE TREATMENT ALGORTHM

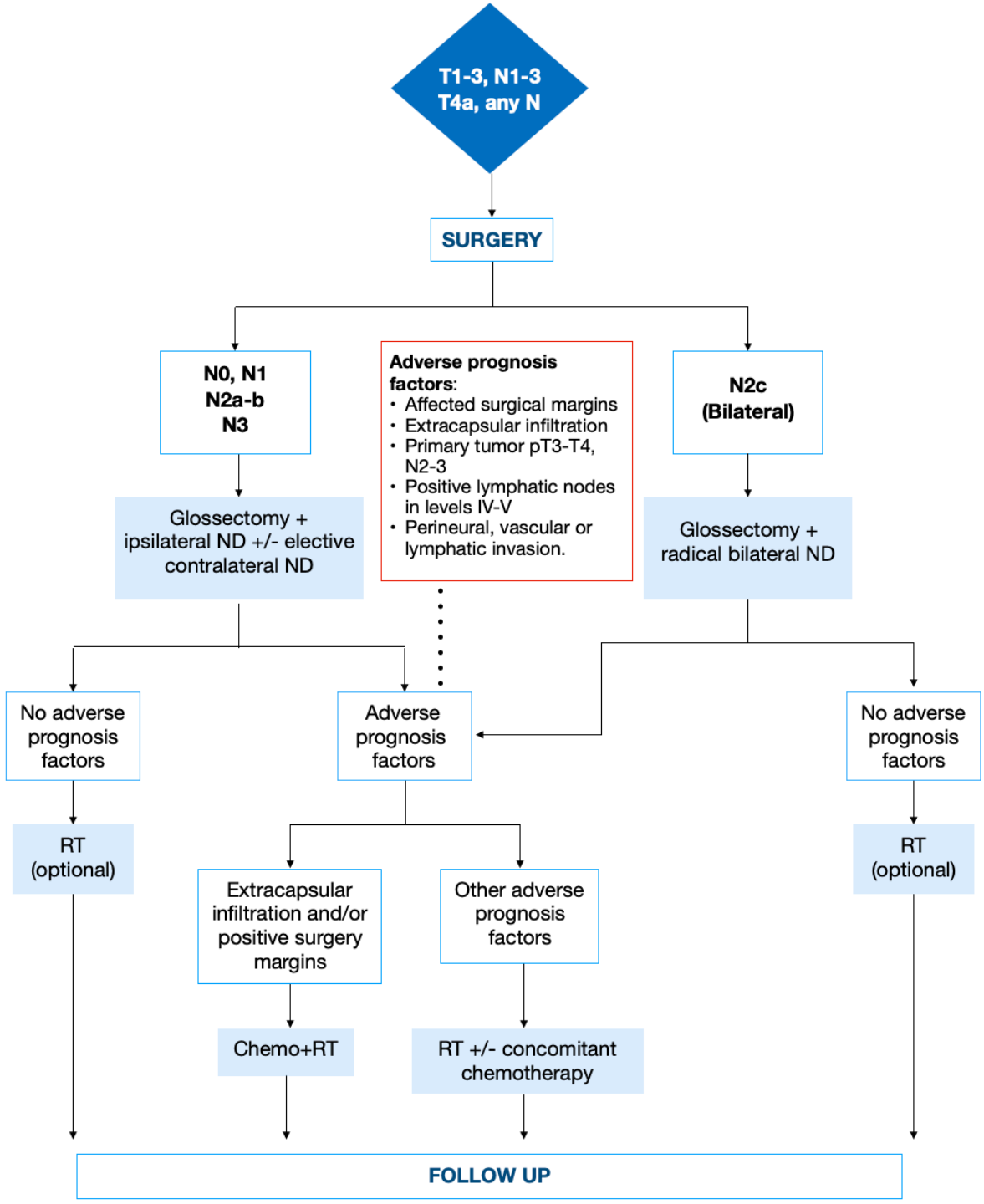


Figure 23. Surgical and adjuvant treatment of advanced stages of SCTC. – ND: Neck dissection;

Adapted from NCCN.

14.5 Annex V: ISCED Scale

The International Standard Classification of Education (ISCED) is the officially recognized framework for facilitating comparisons of education systems across countries. It was initially developed in 1976 by the United Nations Educational, Scientific and Cultural Organization (UNESCO) and has since undergone revisions in 1997 and 2011 (62).

Educational statistics utilizes indicators based on the ISCED 2011 classification system, as detailed below:

ISCED Level	Definition	General classification
ISCED 0	Early childhood education	Low
ISCED 1	Primary Education	
ISCED 2	Lower Secondary Education	
ISCED 3	Upper Secondary Education	Medium
ISCED 4	Post-secondary non-Tertiary Education	
ISCED 5	Short-cycle tertiary education	High
ISCED 6	Bachelor's degree or equivalent tertiary education level	
ISCED 7	Master's degree or equivalent tertiary education level	
ISCED 8	Doctoral degree or equivalent tertiary education level	

Table 15. Table of ISCED scale with general grouping. *Source: The World Data Bank.*

14.6 Annex VI: Informed Consent Form

CONSENTIMENT INFORMAT

Jo, _____ amb document d'identificació personal (DNI/NIE) _____, declaro que:

- He rebut una copia de la fulla de informació per al pacient.
- He llegit i comprès tota la informació facilitada en la fulla de informació per al pacient. He pogut platejar qualsevol dubte que m'ha sorgit, i aquest ha sigut resolt adequadament.
- Estic conforme amb la quantitat d'informació facilitada.
- Comprenc que la meva participació en aquest estudi és voluntària i no remunerada.
- Comprenc els beneficis i riscos que comporta participar en aquest estudi. Comprenc que les meves dades personals seran confidencials i que puc sol·licitar la retirada i eliminació d'aquestes en qualsevol moment de l'estudi.
- Autoritzo que les meves dades i la meva història clínica pugui ser utilitzada per l'equip investigador per a fins relacionats amb l'estudi.
- He entès que puc revocar el meu consentiment informat sobre la participació a l'estudi, sense necessitat d'especificar el motiu i sense que aquest fet afecti a la meva assistència.

En conseqüència, dono lliurament la meva conformitat a participar en l'estudi "Pot una limfogammagrafia prequirúrgica ajudar a la detecció precoç de metàstasi del coll contralateral ocult en el càncer de llengua de cèl·lules escamoses en fase cN0 T3 contralateral no invasiu de la línia mitjana?"

☐ *Accepta* ☐ *Refusa*

Signatura del pacient **Signatura de l'investigador**

Data:

REVOCACIÓ DEL CONSENTIMENT

Jo, _____ amb document d'identificació personal (DNI/NIE)_____ , declaro que la meva decisió d'abandonar l'estudi "*Pot una limfogammagrafia prequirúrgica ajudar a la detecció precoç de metàstasi del coll contralateral ocult en el càncer de llengua de cèl·lules escamoses en fase cN0 T3 contralateral no invasiu de la línia mitjana?*" i, per tant, revoco el consentiment prèviament signat per participar en l'estudi anteriorment mencionat.

Signatura del pacient **Signatura de l'investigador**

Data:

14.7 Annex VII: Patient Fact Sheet

FULL D'INFORMACIÓ PEL PARTICIPANT

NOM DE L'ESTUDI: *Can a presurgical lymphogammagraphy aid in early detection of occult contralateral neck metastasis in contralateral cN0 T3-staged squamous cell tongue cancer non-midline invasive?*

INVESTIGADOR PRINCIPAL: Georgina Ducet

CENTRE DE REFERÈNCIA: Hospital Universitari Doctor Josep Trueta

Benvolgut/a,

Ens dirigim a vostè per convidar-lo a participar en una investigació. Aquest estudi ha rebut l'aprovació del Comitè d'Ètica i Investigació Clínica (CEIC) de l'Hospital Universitari Doctor Josep Trueta, complint amb la legislació actual i els principis de la Declaració d'Hèlsinki.

El propòsit d'aquest document és proporcionar-vos la informació necessària sobre l'estudi perquè pugueu decidir de manera voluntària si voleu participar. Us instem a llegir detingudament aquest document i, en cas de tenir alguna pregunta o dubte, no dubteu a comunicar-vos amb l'investigador principal o l'equip de recerca per aclarir-la.

Quin és l'objectiu de l'estudi?

El present estudi té com a objectiu principal avaluar la incorporació d'una prova basada en la limfogammagrafia com a tècnica de detecció precoç de metàstasis de ganglis cervicals ocultes a l'àrea del coll contralateral al càncer de llengua, que supera els 4 cm però no crossa la línia mitja.

El càncer de llengua representa una neoplàsia en augment en la nostra població. El pronòstic dels pacients afectats per aquest càncer recau sobretot en la presència de ganglis limfàtics cervicals. La presència de metàstasis cervicals redueix la taxa de supervivència als 5 anys al 25%. En estadis més primerencs de la malaltia, aquest procediment és protocol pel diagnòstic i tractament precoç de la malaltia ganglionar.

No obstant això, actualment no existeix cap mesura de detecció precoç per casos més avançats. Els pacients son tractats segons la cirurgia tradicional que no inclou l'abordatge de l'àrea del coll contralateral. Això implica l'assumpció del risc diferit de l'aparició sobtada de ganglis limfàtics i, per tant, l'empitjorament del pronòstic i estat de salut. S'estima que en aquests casos hi ha una taxa de metàstasis ocultes del 20%.

Aquest estudi pretén avaluar aquesta prova per determinar-ne l'eficàcia per detectar aquestes adenopaties. D'aquesta manera, aquest nou abordatge diagnòstic permetrà

identificar aquells pacients que aparentment no presenten adenopaties a l'àrea cervical contralateral encara que realment estan afectes per la malaltia.

Atès que aquesta prova es troba en fase d'estudi, la limfogammagrafia estarà subjecta a control mitjançant la biòpsia del primer gangli que es detecti, coneguda com la biòpsia del gangli sentinella. Els pacients que obtinguin resultats negatius en aquesta biòpsia seran tractats mitjançant cirurgia convencional i se'ls seguirà durant 2 anys després de l'operació.

En conseqüència, només es durà a terme una cirurgia addicional en els pacients que presentin un patró limfogammogràfic que suggereixi metàstasi contralateral i la biòpsia del gangli sentinella del qual sigui positiva. Això és degut a consideracions ètiques, ja que no seria apropiat evitar la cirurgia atès l'elevat risc d'estar afectat per metàstasi amb un resultat histològic maligne i ignorar-ne l'erradicació.

Aquest estudi aportarà evidència que recolzi la futura incorporació de la limfogammagrafia en l'estratègia de detecció precoç de metàstasis ganglionars del coll contralateral en casos de càncer de llengua avançats.

Descripció general i activitats de l'estudi

L'estudi inclourà un total de 118 pacients candidats a glossectomia i buidament ganglionar cervical que hagin estat diagnosticats de càncer de llengua escamós que superi els 4 cm però no envaeixi línia mitja ni estructures profundes. És essencial que els participants estiguin disposats a ser intervinguts quirúrgicament per l'exèresi del tumor primari i els ganglis.

Els individus que acceptin participar a l'estudi, es durà a terme la limfogammagrafia via SPECT/CT uns dies abans de la cirurgia. Aquesta consisteix en una prova d'imatge en què s'administra un radiofàrmac, anomenat tecneci 99, que actua com un traçador per senyalitzar les metàstasis cervicals. Tots els pacients seran operats amb la cirurgia estàndard que consisteix en l'exèresi de la lesió lingual amb marges de seguretat i el buidament dels ganglis del mateix costat del tumor primari.
Segons els resultats de la limfogammagrafia, els pacients es sotmetran a una biòpsia del gangli senyalitzat a la limfogammagrafia el mateix dia de la cirurgia per tal de seleccionar els pacients que requereixin de l'exèresi dels ganglis contralaterals.

L'equip de recerca analitzarà els resultats de la limfogammagrafia i els compararà amb el diagnòstic histològic de la biòpsia de gangli sentinella i, segons el cas també de la peça quirúrgica.

Els següents procediments formen part de la pràctica clínica habitual:

- Estudi d'imatge prequirúrgic de la llengua i el coll (via ressonància magnètica) i d'extensió amb TC a tòrax +/- abdomen. Programació de la cirurgia i visita preoperatòria amb l'equip d'anestèsia.

- Intervenció quirúrgica del tumor primari i els ganglis cervicals i enviament de les peces quirúrgiques al laboratori d'anatomia patològica pel seu anàlisi.
- Visita de control post-quirúrgic per avaluar les ferides quirúrgiques, estat de la llengua, estat general del pacient i comentar resultats de la cirurgia.
- Visites de control trimestrals amb cirurgia maxil·lofacial, oncologia mèdica i oncologia radioteràpica.

La meva participació implica riscos?

La participació en aquest estudi implica riscs baixos degut a que la limfogammagrafia i la biòpsia de gangli sentinella es consideren proves segures. No obstant això, poden haver-hi efectes indesitjats lleus entre els que s'inclou el sagnat lleu o molèsties en l'àrea de l'administració del radiotraçador.

Confidencialitat i tractament de les dades personals

La informació obtinguda serà totalment confidencial, recollida i analitzada anònimament, d'acord amb la *Llei Orgànica de Protecció de Dades de Caràcter Personal i Garantia de Drets Digitals (3/2018) i el Reglament 2016/679 del Parlament i Consell Europeu*.

Les dades personals seran tractades de forma confidencial i només els investigadors de l'estudi hi tindran accés. La informació serà sempre utilitzada amb finalitats de recerca.

Què se'n farà de la informació obtinguda de l'estudi?

Si s'escau per congressos o publicacions, les dades personal seran tractades anònimament per impossibilitar la identificació dels participants.

La participació és obligatòria?

La participació a aquest estudi és completament voluntària. En el supòsit d'acceptar participar, vostè té el dret de revocar el consentiment en qualsevol moment, sense necessitat d'explicar-ne els motius i sense que això provoqui perjudicis en la seva assistència mèdica.

Hi ha compensació econòmica?

No s'ofereix cap compensació econòmica per participar a l'estudi. En cap cas l'estudi suposa cap cost addicional pel pacient.

Contacte en cas de dubtes

Pot posar-se en contacte amb l'investigador principal i els altres membres de l'equip de recerca si al llarg de l'estudi li sorgeixen nous dubtes o necessita més informació. Per qualsevol dubte pot contactar:

maxillofacial-study.dubtes@gmail.com

Secretaria de l'estudi
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